Molekulare und physiologische Analyse redoxregulierter Lichtakklimations-Antworten von *Arabidopsis thaliana*

Dissertation

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1 Einleitung

1.1 *Eukaryota* und *Plantae*

Eukaryoten entwickelten sich aus einem Zweig der Archaeen heraus, wobei der genaue evolutive Hergang ungewiss ist. Es gibt dazu hauptsächlich zwei Hypothesen: entweder entstand der Zellkern, eines der charakteristischen Merkmale eukaryotischer Zellen, durch Einstülpung und Veränderung innerer Membranstrukturen oder durch die Verschmelzung eines Archaeon mit einem Eubakterium (Lake und Rivera, 1994; Margulis *et al.*, 2000). Analysen molekularer Fossilien von Membranlipiden aus dem australischen Pilbara Craton zeigen, dass die Eukaryoten bereits vor etwa 2,7 Mrd. Jahren entstanden sind (Brocks *et al.*, 1999).

Das Reich der Pflanzen umfasst je nach Autor und Definition unterschiedlich viele Gruppen von Ein- und/oder Mehrzellern, die im Besitz von einer oder mehreren Plastiden sind. Generell sind in diese die Moose (*Bryophyta*) und Gefäßpflanzen (*Tracheophyta*) eingeschlossen. Unterschiede in der Einordnung zu den *Plantae* ergeben sich bei den Algen. Häufig wird die grüne Linie der Algen, die Grünalgen (*Chlorophyta*), mit zu den *Plantae* gezählt, die *Rhodophyta*, die rote Linie, jedoch seltener. Nur wenige Autoren ordnen die dritte Linie plastidentragender Eukaryoten, die blaugrünen Algen (*Glaucocystophyta* oder auch *Glaucophyta*), zum Reich der Pflanzen. Im Weiteren werde ich der Einordnung nach Cavalier-Smith (1981) folgen. Dieser Autor unterteilt das Reich der Pflanzen in zwei Unterreiche: *Biliphyta* und *Viridaeplantae*. Die *Biliphyta* bestehen aus der roten und der blaugrünen Linie, während die *Viridiplantae* sowohl die *Chlorophyta*, die *Bryophyta* als auch die *Trachaeophyta* umfassen (http://www.taxonomy.nl).

Die Vorfahren der *Plantae* besaßen bereits einen Zellkern und auch Mitochondrien. Diese Zellorganellen, die ursprünglich freilebende Vorfahren von heute lebenden α-Proteobakterien waren und durch Phagozytose von dem eukaryotischen Vorfahren aufgenommen wurden, entwickelten sich zu intrazellulären Symbionten, die sich vorwiegend auf aerobe Atmung spezialisierten.

Pflanzen entstanden durch ein weiteres singuläres endosymbiontisches Ereignis, in dem ein Vorläufer heutiger Cyanobakterien in die eukaryotische Wirtszelle aufgenommen wurde.

1.1.1 Plastiden und Cyanobakterien haben gemeinsame Vorfahren

Cyanobakterien entstanden vor etwa 2,6 bis 2,4 Milliarden Jahren. Sie sind die Ursache für die Umwandlung der reduzierenden in eine oxidierende Erdatmosphäre, die zwischen 2,4 und 2,2 Milliarden Jahren stattfand. Ein Individuum eines Vorläufers der heutigen Cyanobakterien ging mit einem eukaryotischen, Mitochondrien tragenden Einzeller eine Endosymbiose ein. Nach diesem evolutiv singulären Ereignis (Douglas, 1998) erfolgte genau wie bei der Entstehung der Mitochondrien eine Übertragung von Erbgut vom Endosymbionten zum Wirt hin. Diese Übertragung lässt sich auch heute noch in Laborexperimenten nachweisen und messen. Die Übertragungsfrequenzen sind sehr hoch. Zur Übertragungsweise gibt es zwei Theorien, eine geht von einem Umschreiben von RNA in cDNA aus, welche dann in den Kern exportiert wird, die andere geht davon aus, dass komplette Organellomstücke in den Kern übertragen wurden (Martin, 2003).

In einer evolutionären Übergangsphase mussten noch Kopien funktionstragender Gene in den Organellen verbleiben, bis funktionelle, exprimierbare Kopien im Zellkern integriert waren und deren Genprodukte in die Organellen reimportiert werden konnten. Erst dann waren die Genkopien in den Organellen selbst nicht mehr nötig und gingen im Lauf der Evolution verloren. Doch wurden auch Gene innerhalb der Zelle relokalisiert, so dass die entsprechenden Produkte nicht mehr in den ursprünglichen Endosymbionten gelangten, sondern in ein anderes Kompartiment. Dort übernahmen die Genprodukte eine abgewandelte oder neue Aufgabe. Zum Teil wurden ganze Biosynthesewege verlegt, z.B. die Glykolyse. Weiterhin kam es auch zum Transfer plastidärer und nukleärer Gene in die Mitochondrien (Timmis *et al.*, 2004). Allgemein kann man von „gewürfelter DNA“ und von „gewürfelten Proteomen“ sprechen.

1.1.2 Die Zelldifferenzierung ging einher mit der Organellendifferenzierung

Die Pflanzen entwickelten sich im Meer und blieben dort für die nächsten 1,5 Mrd. Jahre. Die ersten fossilen Landpflanzen sind erst im späten Ordovizium (vor 488 bis vor 444 Millionen Jahren) nachweisbar. Zur Besiedlung des Landes entwickelten die Pflanzen verschiedene Gewebe und Organe. Dies erforderte Zelldifferenzierungsprozesse, die auch Auswirkungen auf die Organellen zeigten.

Je nach Gewebetyp und Funktion entwickeln sich die Zellen heutiger Landpflanzen verschieden. Diese Differenzierung wird durch die Aktivierung und Inaktivierung verschiedener Gene des Nukleus gesteuert und somit durch differentielle Genexpression. Die Organellen werden ebenfalls durch anterograde, vom Nukleus ausgehende, Kontrollprozesse den Gewebsfunktionen angepasst. Am deutlichsten tritt dies bei der Differenzierung der Plastiden hervor.

Plastiden werden nicht-mendelnd vererbt. Bei Angiospermen geschieht dies über die Eizelle, wogegen die Plastiden der Pollen abgebaut werden. Sie liegen im jungen Embryo als Proplastiden vor, die sich in Abhängigkeit von Organ und Gewebelage entwickeln: So sind Chloroplasten die Orte der Photosynthese und in den Sprossachsen und Blättern von höheren Pflanzen zu finden, wobei sie dort im Mesophyllparenchym und in den Spaltzellen vorkommen. Als Gerontoplasten bezeichnet man alternde Chloroplasten. Amyloplasten sind Orte der Stärkespeicherung in der Wurzel, Chromoplasten sind häufig die farbgebenden Komponenten von Blüten oder Früchten. Leukoplasten dienen als Fettspeicher. Als Etioplasten bezeichnet man Plastidenformen, die sich während der Skotomorphogenese aus Proplastiden entwickeln (Buchanan *et al.*, 2000).

1.2 Horizontaler Gentransfer führt zu anterograder Kontrolle

Es ist wahrscheinlich, dass der Transfer von Organell-DNA zum Kern hin erst nach Eintreten der Endosymbiose erfolgte (siehe auch 1.1.1). Da der Nukleus eine eukaryotische Transkriptionsmaschinerie verwendet, die transferierten Genkopien jedoch einem prokaryotischen Organismus entstammen, musste der Transfer vielfach erfolgen, um zu gewährleisten, dass die Genkopie in richtiger Orientierung und im richtigen Leseraster hinter einem eukaryotischen Promotor inserierte. Weiterhin bedurfte es Sortiermechanismen, um die Produkte der Genkopien wieder ins Organell transportieren zu können. Sobald diese mehrstufige Entwicklung der Translokation vollzogen war, konnte auf das Originalgen des

Organells verzichtet werden. Somit führte dieser Transfer zu einer kerngesteuerten Entwicklung und Funktionalisierung des zelleigenen Organellsatzes, was man als anterograde Kontrolle bezeichnet. Da Plastiden in funktionellem Zustand mehrere tausend verschiedene Proteine beinhalten, wird der Großteil der Proteine in die Plastiden importiert, d.h. die anterograde Kontrolle hat einen sehr starken Einfluß auf die plastidäre Differenzierung.

Doch selbst zwei Milliarden Jahre nach dem endosymbiontischen Ereignis sind noch funktionstragende Gene ihrer Vorfahren sowohl in Mitochondrien als auch Plastiden vorhanden. In Mitochondrien wurden zusätzlich Kopien ursprünglich nukleärer oder plastidärer Gene gefunden.

So kodiert das Organellgenom der Plastiden in höheren Pflanzen für bis zu 120 Genprodukte (Goldschmidt-Clermont, 1998), die in ihrer Zusammensetzung relativ konserviert sind. Hinzu kommt, dass sie gewissen Funktionsclustern angehören. Dazu zählen Gene für den Aufbau des prokaryotischen Transkriptionsapparates und Komponenten des plastideneigenen Proteinbiosyntheseapparates. Weiterhin werden, z.B. in den Plastiden von *Arabidopsis*, einige wenige Enzyme kodiert, Untereinheiten des Nitratdehydrogenase (NDH)-Komplexes, einige Assemblierungsfaktoren für Photosystem I und Zentrumskomponenten der vier integralen Multiproteinkomplexe der Thylakoidmembran.

Für den Verbleib von DNA in den Organellen gibt es verschiedene Theorien, die ich nachfolgend kurz aufführe (zur Übersicht siehe Allen, 2003).

Einige Annahmen gehen davon aus, dass der Prozess des Gentransfers noch nicht vollendet ist. Dafür spricht, dass das zytoplasmatische Transkriptions- und Translationssystem jegliche Proteine, so auch prokaryotische, herstellen kann. Weiterhin deuten vorhandene Daten darauf hin, dass das evolutionäre Ziel der eukaryotischen Pflanzenzelle eine Dekompartimentierung genetischer Information bei gleichzeitiger Aufrechterhaltung der Kompartimentierung physiologischer Prozesse ist (Herrmann und Westhoff, 2001). Gegen diese Hypothese spricht, dass die in den Organellen verbliebenen Gene nicht zufällig, sondern über ein breites Artenspektrum, welches sowohl höhere Pflanzen als auch Algen umfasst, in recht konservierter Zusammensetzung vorliegen und gleichen Funktionsgruppen angehören (Barbrook *et al.*, 2001). Weiterhin konnte gezeigt werden, dass der horizontale Gentransfer zwischen Organellen und Nukleus noch heute stattfindet, jedoch sehr schnell von statten geht (Henze und Martin, 2001; Timmis *et al.*, 2004). Durch das *Arabidopsis Genome Project* wurde das Kerngenom von *Arabidopsis thaliana* vollständig sequenziert (AGI, 2000). Eine Analyse der Sequenzen zeigt, dass bereits alle in Plastiden vorhandenen Genkopien im Nukleus existieren, dort jedoch nicht exprimiert werden. Diese Fakten bestärken die Vermutung, dass ein Grund für den Verbleib spezifischer Gene in energetischen Organellen vorliegen muss.

Einige ältere Annahmen gehen von einem Stop des Gentransfers aus, da eine mögliche Fehllokalisation der Genprodukte zu einem Tod des Organismus führt und somit einen Selektionsdruck für einen Verbleib der Gene in den Organellen darstellt (Bogorad, 1975; Von Heijne, 1986). Mittlerweile kennen wir jedoch die Lokalisationsmechanismen und ihre Spezifität (Jarvis und Soll, 2001; Schatz, 1998), weshalb diese Theorien ausgeschlossen werden können. Ergänzt werden muss ein evolutionärer Spezialfall. Es ist bekannt, dass der Gentransfer nach Lyse von Plastiden stattfindet, da nur so die hohen Translokationsfrequenzen erreicht werden können. Bei Einzellern, die nur eine Plastide

besitzen, würde eine Lyse somit zum Absterben des Organismus führen (Barbrook *et al.*, 2006). Dennoch konnten bei *Chlamydomonas* Translokationsfrequenzen gemessen werden, diese waren jedoch erheblich geringer als z.B. bei Tabak. Ein möglicher Transfer bei diesen Organismen könnte somit die Aufnahme plastidärer DNA eines anderen Individuums sein.

Weitere Hypothesen postulieren, dass gewisse Genprodukte nicht in die Organellen importiert werden können. Als mögliche Importschranken werden die Hydrophobizität (Claros *et al.*, 1995; Von Heijne, 1986), die Anzahl der transmembranalen Domänen (Popot und Devitry, 1990; Wollman *et al.*, 1999) und/oder die Tertärstruktur in Verbindung mit einem oder mehreren Cofaktoren genannt. Diese Annahmen stehen jedoch in Kontrast zu einigen Gegenbeispielen. So erklärt die Hydrophobizität nicht, warum die große Untereinheit der Rubisco, ein wasserlösliches Protein, plastidär kodiert ist. Demgegenüber werden die Apoproteine der Lichtsammelkomplexe (*light harvesting complex* II/I, LHCII/I) von Photosystem II (PSII) und Photosystem I (PSI) im Nukleus kodiert. Diese besitzen zwar nur drei transmembranale Domänen, doch werden ebenso ADP-ATP-Transporter (AACs), welche bis zu zwölf transmembranale Domänen besitzen (Tjaden *et al.*, 1998), von Mitochondrien und Chloroplasten im Nukleus kodiert. Dies wiederspricht somit der These, dass Gene in den Organellen verbleiben, da deren Genprodukte zu viele transmembranale Domänen besitzen. Proteinimport in Organellen erfolgt meist über Chaperone (Jackson-Constan *et al.*, 2001). Für komplexe Strukturen, die nicht entfaltet werden können, da sonst ein cytotoxisches Potential durch freigegebene Cofaktoren auftreten könnte, wäre ein Import über den TAT-Mechanismus (*Twin*-Arginin-Translokase) (Sargent *et al.*, 2002) denkbar. Weiterhin deuten Indizien auf ein vesikuläres Transportsystem (Westphal *et al.*, 2001) in Chloroplasten hin, welches ebenso Tertiärstrukturen transportieren könnte, ohne sie vorher zu entfalten.

Andere Annahmen betreffen mögliche genetische Inkompatibilitäten zwischen Organellgenen und eukaryotischer Transkriptionsmaschinerie. So gibt es in den Mitochondrien durch den Wobble-Effekt eine unterschiedliche Triplettnutzung zwischen den Großgruppen der Tiere, Pilze und Pflanzen. Durch die nötige und starke Konservierung der Genprodukte wird vermutet, dass durch einen stärkeren Wobble-Effekt eine mögliche genetische Veränderung keine Auswirkung auf das Genprodukt besitzt und somit von Vorteil ist. Andererseits kann solch eine Spezialisierung nicht den generellen Verbleib von Genen in der Nähe eines Systems erklären, welches Radikale erzeugt. Weshalb mindestens ein weiterer Grund für den Verbleib vorliegen muss. Für verbliebene plastidäre Gene wird ebenso vermutet, dass sie ungewöhnliche Tripletts aufweisen und somit diese einem Transfer entgegenstehen (Howe *et al.*, 2000). Plastiden nutzen keine ungewöhnlichen Aminosäuren (Allen, 2003), weshalb diese Triplettnutzung als Folgeeffekt angesehen werden kann und nicht als generelle Ursache des Verbleibs der Organellgene.

Die CORR (*co-location of genes and gene product for evolutionary continuity of redox regulation of gene expression*)-Hypothese besagt, dass Elektronen- und Protonentransport eine Expressionskontrolle über jene Gene ausübt, die Komponenten bilden, welche direkt auf das Redoxgleichgewicht wirken oder indirekt darauf Einfluss nehmen können (Allen, J. F., 1993; Allen, 2003). Diese regulatorische Kopplung ist unverzichtbar, um auf Umwelteinflüsse zu reagieren. Würden diese Gene in den Nukleus transferiert werden, so wäre die Regulationskopplung unterbrochen. Somit kann man Organellen als autonome Systeme in Hinsicht auf die Regulation ihrer Redoxhomöostase betrachten. Doch auch diese

letzte Hypothese über den Verbleib von genetischem Matierial erklärt nicht alle beobachteten Fakten. Mitochondrien haben ein Genom, welches, je nach betrachtetem Organismus, zwischen drei und neunzig Genprodukte kodieren kann. Lediglich Cytochrom b ist in allen vorhanden. Zwar ist Cytochrom b essentiell für die Respiration, doch besteht die Respirationskette aus mehr Elementen. Laut der CORR-Hypothese müssten zumindest die Elemente in jenen Mitochondrien enthalten sein, welche der Respiration dienen. Weiterhin ist aus *Chlamydomonas reinhardtii* bekannt, dass kernkodierte Gene ebenso schnell exprimiert werden können wie organellkodierte Gene (Zerges, 2002). Dies deutet darauf hin, dass nicht die Geschwindigkeit der Expression für den Verbleib wichtig ist, sondern die Kopplung der Expression dieser Gene an das Redoxgleichgewicht. Die CORR-Hypothese kann nicht erklären, warum nach sekundärer oder tertiärer Endosymbiose, wie wir sie z.B. in *Euglena viridis* vorfinden, im Nukleomorph noch genetisches Material vorhanden ist. Schließlich ist an diesen nicht direkt ein Elektronen- oder Protonentransport gebunden.

1.3 Kommunikation zwischen Nukleus und Plastiden

In pflanzlichen Zellen kann die Anzahl der Plastiden zwischen Arten und Geweben stark schwanken. Die Grünalge *Chlamydomonas reinhardtii* besitzt nur eine Plastide, während eine Mesophyllzelle höherer Pflanzen mehrere Dutzend beinhalten kann. Da pro Plastide mehrere Plastome vorliegen, steht eine große Anzahl plastidärer Genkopien einer geringen Zahl nukleärer Genkopien gegenüber. In *Arabidopsis thaliana* kann ein bis zu 10.000-facher Überschuß an plastidären Kopien vorliegen. Da funktionelle Multiproteineinheiten, wie z.B. die Ribulose-1,5-bisphosphat-carboxylase/-oxygenase (Rubisco) oder die Photosynthesekomplexe, sowohl aus kernkodierten als auch aus plastidär kodierten Untereinheiten bestehen, ist es für die Zelle unabdingbar, nukleäre und plastidäre Genexpression zu koordinieren, um die Substöchiometrie der Untereinheiten zu balancieren. Die Koordination setzt eine Signalgebung zwischen den Kompartimenten voraus.

Diese Kommunikation beeinflusst sowohl in den Plastiden als auch im nukleo-zytosolischen Kompartiment die Transkription (Leister, 2005; Pfannschmidt *et al.*, 1999a), posttranskriptionelle Vorgänge (Rochaix, 2001) und Translationsmechanismen (Choquet und Wollman, 2002). Eine Funktionsregulation findet nicht nur auf Ebene der Genexpression statt, sondern es konnte gezeigt werden, dass posttranslationelle Modifikationen (Aro und Ohad, 2003; Buchanan *et al.*, 1994; Wollman, 2001), strukturelle Reorganisation (Niyogi, 1999), metabolische Regulation (Paul und Peliny, 2003) und ein Einfluß auf Entwicklungsvorgänge (Rodermel, 2001) betroffen sind. Die Koordination dieser Prozesse erfolgt sowohl durch anterograde Signale als auch durch die Organellen selbst, welche Informationen an die anderen Kompartimente der Zelle abgeben (retrograde Signalgebung).

Bisher konnten sechs plastidäre und zwei mitochondriale retrograde Signalquellen identifiziert werden. Zu den plastidären zählt man Metabolite des Kohlenstoffstoffwechsels (Rolland *et al.*, 2002), Intermediate des Tetrapyrrolsyntheseweges (Brown *et al.*, 2001; Rodermel und Park, 2003; Strand, 2004; Strand *et al.*, 2003), reaktive Sauerstoffspezies (Apel und Hirt, 2004), den Redoxzustand des Plastochinonpools (Pfannschmidt, 2003), die plastidäre Translation (Adamska, 1995; Oelmüller *et al.*, 1986; Sullivan und Gray, 1999; Yoshida *et al.*, 1998) und den Redoxzustand des Cytochrom b_6f-Komplexes (Shao *et al.*, 2006). Als mitochondriale Signalquellen hat man Häm (Guarente und Mason, 1983; Pfeifer *et*

al., 1989) und die mitochondriale Translation (Pesaresi *et al.*, 2006) identifizieren können, wobei die Funktion des Häms noch nicht in Pflanzen gezeigt werden konnte (Surpin *et al.*, 2002). Es gibt Indizien dafür, dass diese Signalwege miteinander und mit den Transduktionswegen der zytoplasmatischen Lichtrezeptoren interagieren (Lopez-Juez *et al.*, 1998; Shao *et al.*, 2006; Vinti *et al.*, 2005) und ein Signalnetzwerk bilden. Doch ist nur sehr wenig darüber bekannt, wie diese Signale perzipiert und weitergeleitet werden.

1.4 Der Photosyntheseapparat – Generator und Sensor

Wohl keiner der Organellen in Pflanzen wurde soviel Aufmerksamkeit geschenkt wie den Chloroplasten, da in ihnen jener Prozess abläuft, welcher die energetische Grundlage für alle heterotrophen Lebewesen bildet: die Photosynthese.

Der Photosyntheseapparat ist in den Thylakoiden, spezielle Membranstrukturen der Chloroplasten, eingebettet. Er besteht im Wesentlichen aus vier transmembranalen multimeren Proteinkomplexen: Photosystem II (PSII), dem Cytochrom b_6f-Komplex (Cyt b_6f-Komplex), Photosystem I (PSI) und der ATP-Synthase. Die Thylakoide werden ihrem Erscheinungsbild nach in Grana- und Stromathylakoide unterschieden. PSII ist vorwiegend in den Grana-, PSI in den Stromathylakoiden zu finden. Der Cyt b_6f-Komplex ist in beiden Thylakoidstrukturen lokalisiert. Die ATP-Synthase befindet sich hauptsächlich in den Stromathylakoiden und in den peripheren Bereichen der Granathylakoide (Blankenship, 2002).

Photosystem II und I sind energetisch gekoppelt. Diese Kopplung wird durch die Elektronenüberträger Plastochinon (PQ), Plastocyanin (PC) und den Cyt b_6f-Komplex realisiert. PQ transportiert zwei Elektronen von PSII entlang der Membran zum Cyt b_6f-Komplex und PC transportiert je ein Elektron vom Cyt b_6f-Komplex zu PSI. Während des Transportes wird über die Thylakoidmembran zwischen Stroma und Lumen ein Protonengradient aufgebaut. Zum einen transportiert PQ jeweils zwei Protonen pro zwei Elektronen, während der Cyt b_6f-Komplex durch den Q-Zyklus wiederum Protonen aus dem Stroma aufnimmt und ins Lumen abgibt. Weiterhin werden durch die Photolyse von Wasser am Wasserspaltungsapparat des PSII Protonen und Sauerstoff im Lumen freigesetzt. Der Wasserspaltungsapparat, bestehend aus PsbO, PsbP, PsbQ und PsbR, liefert Elektronen über einen Tyrosinrest des D1-Proteins an das Reaktionszentrum von PSII und füllt somit die durch die Lichtreaktion entstandene Elektronenlücke (Buchanan *et al.*, 2000).

Die Antennenkomplexe, die sowohl PSII als auch PSI umgeben, sammeln und leiten die Energie für die primäre Lichtreaktion der Photosystemzentren und enthalten den Hauptteil der akzessorischen Pigmente des Photosyntheseapparates. Letzteres hat man bereits in den dreißiger Jahren des 20. Jahrhunderts durch Gabe von kurzen Lichtblitzen feststellen können (Emerson und Arnold, 1932a, b). Sie sind für die Absorption des Lichts und die Weiterleitung der Energie an die Reaktionszentren der Photosysteme verantwortlich. Die wichtigsten theoretischen Eckpunkte zu diesen Energieübertragungsmechanismen wurden von Gaffron und Wohl (Gaffron und Wohl, 1936), Franck und Teller (Franck und Teller, 1938) und Förster in den vierziger Jahren des 20. Jahrhunderts (Förster, 1965) geschaffen. Generell besitzen beide Photosysteme einen inneren immobilen Antennenkomplex. PSII besitzt zusätzlich eine äußere Antenne, die sowohl in ihrer Größe und Qualität variieren und auch zwischen den Photosystemen verschoben werden kann. Dieser Antennenkomplex wird

allgemein als LHCII bezeichnet und besteht aus trimeren Untereinheiten, welche in unterschiedlicher Zusammensetzung aus den Monomeren Lhcb1, Lhcb2 und Lhcb3 gebildet werden. LHCII ist über die innere Antenne des PSII verbunden, welche wiederum über die *core*-Antenne mit dem Reaktionszentrum verbunden ist. Bei niedriger Lichtstärke ist der Antennendurchschnitt, also die Anzahl der mit PSII verbundenen LHCII-Trimere, größer. Zieht man Pflanzen bei höheren Lichtstärken an, nimmt der Antennendurchschnitt ab. Wechselt man jedoch an Schwachlicht akklimatisierte Pflanzen in stärkeres Licht, so verringert sich der Antennendurchschnitt höchstens über einen längeren Zeitraum durch das Wachstum, was darauf hindeutet, dass es keine besonderen Abbaumöglichkeiten der stabilen LHCII-Trimere gibt (Melis, 1998).

Durch die Absorption und vektoriellen Energietransport innerhalb der Photosysteme wird ein spezifisches Chlorophyllpaar (das so genannte *special pair*) im Zentrum der Komplexe angeregt, was eine gerichtete Abgabe eines Elektrons aus dem jeweiligen zentralen Chromophorenpaar zur Folge hat. Durch eine Reihe von Reduktions- und Oxidationsreaktionen entstehen Reduktionsäquivalente wie Nikotinamidadenin-dinukleotidphosphat ($NADPH^+$) und, wie oben erläutert, ein Protonengradient. Die ATP-Synthase, einer der vier großen Proteinkomplexe der Thylakoidmembran, nutzt den entstandenen Protonengradienten zur Herstellung von Adenosintriphosphat (ATP) aus Adenosindiphosphat (ADP) und anorganischem Phosphat (P_i). Das erzeugte ATP und auch die Reduktionsäquivalente werden u. a. zur Fixierung von Kohlendioxid (CO_2) und der Synthese von Zuckern genutzt und stellen damit die Grundlage des pflanzlichen Energie- und Baustoffwechsels dar (Buchanan *et al.*, 2000; Wildner und Häder, 1999).

Pflanzen sind sessile Organismen und haben keine Möglichkeit ungünstigen Umweltbedingungen auszuweichen. Aus diesem Grund besitzen sie eine Reihe von Perzeptionssystemen, um auf fluktuierende Bedingungen optimal reagieren zu können. Eines dieser Rezeptorsysteme bilden die Plastiden, da sie neben den zytosolischen Photorezeptoren Licht absorbieren. Unterschiedliche Lichtstärken beeinflussen den linearen Elektronentransport, den Redoxzustand der Komponenten der Elektronentransportkette und den der Endakzeptoren. Durch die unterschiedlichen Absorptionseigenschaften von PSII und PSI kann der photosynthetische Prozess Lichtqualitätsunterschiede perzipieren. Da die Fixierung des Kohlendioxides im plastidären Stroma stattfindet, werden dort Mangelsituationen an CO_2, durch die fehlende Aktivierung der Ribulose-1,5-Bisphosphat-Carboxylase/Oxigenase (Rubisco) mittels Carbamylierung, wahrgenommen und die Plastide kann auf diese reagieren (Wildner und Häder, 1999).

Während der Lichtphase der Photosynthese können bei der primären Reaktion und den auf sie folgenden Elektronen- und Protonentransportprozessen hochreaktive Radikale gebildet werden, welche sowohl auf Chloroplasten als auch auf die Pflanzenzellen selber toxisch wirken. Die Entstehung der Radikale häuft sich bei einem Anstieg der Lichtstärke oder bei gleichbleibender Lichtstärke und abfallender Temperatur. Diese Bedingungen können zu einer Bildung von Triplettchlorophyllen und von reaktiven Sauerstoffspezies (ROS) führen. So erfolgt bei einer Überanregung der photosynthetischen Elektronentransportkette ein erhöhter Elektronenübergang von PSI auf Sauerstoff und in weiterer Folge wird H_2O_2 gebildet. Weiterhin erfolgt Photorespiration, wodurch verstärkt Phosphoglykolat gebildet wird. Das Phosphoglykolat wird in die Peroxisomen exportiert und dort unter Bildung von

H_2O_2 umgesetzt. Wasserstoffperoxid, Peroxide im Allgemeinen, Singulett-Sauerstoff, das Superoxid-Radikal und das Hydroxyl-Radikal werden zu den ROS gezählt (Apel und Hirt, 2004).

Da die Photosynthese sowohl Energiequelle als auch Ursprung vieler hochreaktiver Substanzen ist, unterliegt sie verschiedenen Regulationsmechanismen. Diese können sowohl Puffer (wie Glutathion) als auch Detoxifikationssysteme (wie der Xantophyllzyklus) sein. Doch existieren auch Mechanismen, die die Primärreaktion der Photosynthese beeinflussen. Diese können kurzzeitig strukturelle Umlagerungen und Modifikationen funktioneller Gruppen hervorrufen, sowie auf längere Zeit den Abbau und die Neusynthese einzelner Komponenten der Elektronentransportkette beeinflussen. Die Neusynthese von Proteinen kann auf verschiedenen Ebenen reguliert werden: Transkriptionsaktivität, RNA-*Splicing*, RNA-Modifikation, RNA-Stabilität, Translationsinitiation, Proteinprozessierung, posttranslationelle Modifizierung und Transportraten für plastidäre Proteine und Proteinuntereinheiten, die im Nukleus kodiert werden. Doch sind noch andere Regulationsmechanismen denkbar, wie die Entdeckung der *Riboswitches* (Breaker, 2004; Nahvi *et al.*, 2002) zeigt.

1.4.1 Klärung des Begriffs Redoxkontrolle

„Redoxreaktionen sind chemische Reaktionen, bei denen eine Übertragung von Elektronen oder Protonen zwischen Molekülen erfolgt. Reduktion heißt, dass ein Elektronenakzeptor ein oder mehrere Elektronen oder Wasserstoffatome hinzugewinnt. Oxidation heißt, dass ein Elektronendonor ein oder mehrere Elektronen oder Wasserstoffatome abgibt. Redoxstatus oder auch Redoxzustand ist der Reduktions- oder Oxidationszustand eines bestimmten Moleküls. Redoxkontrolle eines biologischen Vorgangs bezeichnet nun eine molekulare Antwort in Abhängigkeit von dem Redoxzustand eines oder mehrerer Moleküle, die in diesen biologischen Vorgang involviert sind.“ (aus dem Englischen übersetzt, Pfannschmidt *et al.*, 2001a).

In diese Definitionen kann man die der Redoxsignale einreihen. Ein Redoxsignal ist jenes oder jene Moleküle, deren Redoxzustand eine Redoxkontrolle hervorrufen. Der Redoxzustand eines Molekülpools wird durch die Nernst - Gleichung beschrieben:

$$E = E_m + \frac{RT}{zF} \cdot \ln \frac{a_{ox}}{a_{red}}$$

Gl. 1.4.1a: Nernst - Gleichung. E: Redox Potential, E_m: Standardpotential, R: Universelle Gaskonstante, T: absolute Temperatur, z: Anzahl der übertragenen Elektronen (Äquivalentzahl), F: Faraday-Konstante, a: Aktivität des entsprechenden Redoxpartners. In erster Formulierung wurden statt der Aktivitäten die Konzentrationen verwendet. Doch treffen diese nur in idealen Lösungen zu, weshalb man zur Verwendung der Aktivitäten, die Umgebungseinflüsse des Lösungsmittels beachten, überging.

Die Nernst-Gleichung gibt ein relatives Potentialgefälle eines Redoxpaares gegenüber einer Wasserstoffhalbzelle unter Standardbedingungen an. Je positiver dieser Wert ist, umso leichter gibt ein Redoxpaar Elektronen frei.

1.4.2 Der photosynthetische Elektronentransport erzeugt Redoxsignale

Durch den photosynthetischen Elektronentransport werden Elemente der Elektronentransportkette gezielt oxidiert oder reduziert. Der Redoxzustand einzelner Elemente der Elektronentransportkette liefert somit Auskunft über die Auslastung der Elektronentransportkette. Doch auch Moleküle, die durch den photosynthetischen Elektronentransport auf der Akzeptorseite von Photosystem I reduziert werden, können als Signalquelle, also als ein Redoxsignal, fungieren. Somit kann man zwischen Redoxsignalen unterscheiden, die innerhalb der Elektronentransportkette direkt erzeugt werden (perzeptionelle Redoxkontrolle) und Redoxsignalen, welche hinter dem sensorischen System erzeugt werden (transduktionelle Redoxkontrolle) (Pfannschmidt *et al.*, 2001a). In der Literatur gibt es eine Reihe von Beispielen.

1.4.2.1 Transduktionelle Redoxkontrolle

Diese Form der Redoxkontrolle liegt vor, wenn stromale Elemente, die durch den Photosyntheseapparat reduziert werden können, signalgebende Wirkung besitzen. Sie kommt sowohl in höheren Pflanzen als auch in Algen vor. Ein altbekanntes Beispiel ist Thioredoxin, welches über Ferredoxin reduziert werden kann. Thioredoxin ist eine Disulfidoxidoreduktase, welche Enzymaktivitäten mittels Reduktion von Disulfidbrücken regulieren kann (Motohashi *et al.*, 2001). In höheren Pflanzen liegen zwei Isoformen vor: Thioredoxin f und Thioredoxin m. Beide unterscheiden sich weder in ihrem aktiven Zentrum noch in ihrer Tertiärstruktur, dennoch zeigen beide Thioredoxinformen unterschiedliche Substratspezifitäten für eine große Anzahl an Proteinen. Man nimmt an, dass die Ladungsverteilungen um das jeweilige aktive Zentrum herum verschieden sind. Die klassischen Beispiele der Thioredoxinkontrolle sind vier Enzyme des Calvin-Benson-Zyklus. Dazu gehören die Glyzerinaldehyd-3-phosphatdehydrogenase (GAPDH, (Baalmann *et al.*, 1995), die Fruktose-1,6-bisphosphatase (Clancey *et al*., 1987), die Seduheptulose-1,7-bisphosphatase (Cadet *et al.*, 1987) und die Phosphoribulokinase (Wolosiuk und Buchanan, 1978). Letztere und die CF_1 Untereinheit der plastidären ATP-Synthase werden über Typ f Thioredoxin reguliert. Thioredoxin vom m-Typ reguliert z.B. die NADPH abhängige Malatdehydrogenase (Miginiac-Maslow *et al.*, 2000). In den letzten zehn Jahren wurden eine Reihe weiterer Substrate identifiziert, die durch Thioredoxine reguliert werden. Ebenfalls bei Stressantworten scheinen die plastidären Thioredoxine eine entscheidende Rolle zu spielen, wie die Arbeiten von Carlberg *et al.* (Carlberg *et al.*, 1999) und Baier und Dietz (Baier und Dietz, 1999) zeigen. Carlberg *et al.* zeigten, dass thylakoidassoziierte Kinasen bei Starklicht reguliert werden, während Baier und Dietz die physiologische Bedeutung für 2-Cys-Peroxiredoxine für antioxidative Reaktionen und deren Regeneration durch Thioredoxine zeigten (König *et al.*, 2003). Doch nicht nur auf Ebene der Aktivitätsregulation metabolischer Enzyme kann Thioredoxin wirken. So wurden in *in vitro* Experimenten Hinweise gefunden, dass Thioredoxin die Bindung von translationsaktivierenden Proteinkomplexen an die 5'-Region der *psbA* mRNA in *Chlamydomonas reinhardtii* reguliert (Danon und Mayfield, 1994; Mayfield *et al.*, 1994). Weitere Beispiele für einen möglichen Einfluss auf die plastidäre Genexpression sind das RB60 Protein (Kim und Mayfield, 1997) und Untersuchungen an der *Chlamydomonas*-Mutante MU7 (Salvador und Klein, 1999).

Neben den Thioredoxinen kann ebenfalls der Redoxzustand des Glutathions (GSH) durch den photosynthetischen Elektronentransport beeinflusst werden, sei es direkt oder über antioxidative Reaktionen mit ROS. GSH kommt in großen Mengen in den Chloroplasten vor und deshalb wird dieser Substanz eine Pufferfunktion zugeschrieben. Es gibt durch *in vitro* Experimente Hinweise darauf, dass auch GSH ein Redoxsignal ist. An der plastidären Transkriptionskinase (PTK) aus *Sinapis alba*, welche Teile des plastidären Transkriptionsapparates phosphorylieren kann, wurde *in vitro* gezeigt, dass deren Aktivität durch reduziertes GSH gesteigert werden kann (Baginsky *et al.*, 1997; Baginsky *et al.*, 1999). Glutathion ist ein Massenpeptid der Chloroplasten, da es eine wesentliche Rolle im Wasser-Wasser-Zyklus spielt (Asada, 1999). Dort regeneriert die Dehydroxyascorbinsäurereduktase Dehydroxyascorbinsäure zu Ascorbinsäure unter Oxidation von GSH. Das entstandene GSSG (oxidierte Form des Glutathions) wird in einer Folgereaktion durch die GSH-Reduktase reduziert, wobei NADPH als Elektronendonor dient.

Ebenfalls signalvermittelnd wirkt die Entstehung von ROS durch die Photoreduktion von Sauerstoff an der Akzeptorseite von PSI und ist somit eine Form der transduktionellen Redoxkontrolle. Die Signalüberträger sind vermutlich die ROS, welche durch Folgereaktionen der Sauerstoffphotoreduktion entstehen, da angenommen wird, dass z.B. Wasserstoffperoxid (H_2O_2) ungehindert die Plastiden verlassen kann. Wasserstoffperoxid wirkt auch als *second messenger* bei systemischen Stressantworten (Karpinski *et al.*, 1999) und ebenfalls als Plastidensignal, welches die Expression nukleärer Gene moduliert (Gray *et al.*, 2003).

1.4.2.2 Perzeptionelle Redoxkontrolle der Photosynthese

Redoxregulation durch Licht ist unter photosynthetischen Organismen sehr weit verbreitet. Die CORR-Hypothese (siehe 1.3) geht von einer evolutionären Konservierung des Mechanismus aus (Allen, 2003). So finden wir lichtgesteuerte redoxkontrollierte Prozesse sowohl in Cyanobakterien als auch in Chloroplasten einzelliger Algen und denen höherer Pflanzen. In Experimenten, die der Aufklärung der Signalquellen und der Auswirkungen photosynthetischer Redoxkontrolle dienten, wurden verschiedene Lichtqualitäten, Lichtstärken und Temperaturen genutzt, um den photosynthetischen Elektronentransport zu modifizieren. Inhibitoren wie 3-(3',4'-Dichlorphenyl)-1,1'-dimethylharnstoff (DCMU) und 2,5-Dibromo-3-methyl-6-isopropyl-p-benzochinon (DBMIB) (Trebst, 1980), die spezifisch photosynthetische Elektronenübertragungen hemmen, können eingesetzt werden, um photosynthetische Redoxsignale von Phytochrom, Cryptochrom und Phototropin vermittelter Lichtperzeption und Signalgebung zu unterscheiden.

Bisher konnten zwei Komponenten der Elektronentransportkette identifiziert werden, von denen Redoxsignale ausgehen: Plastochinon und der Cyt b_6f-Komplex. Die Auswirkungen von diesen Redoxsignalen sind jedoch verschieden. So beeinflusst der Redoxzustand des PQ-Pools die Energieverteilung zwischen den Photosystemen durch eine Kurz- und eine Langzeitantwort, während der Cyt b_6f-Komplex einen Einfluss auf die Expression einiger nukleärer Gene hat, welche für Enzyme der Chlorophyllbiosynthese kodieren (Shao *et al.*, 2006). Der genannte Einfluss des Cyt b_6f-Komplex wurde erst 2006 entdeckt und ist bisherigen Erkenntnissen nach unabhängig vom Redoxzustand des Plastochinonpools. Die genaue Signalwirkung ist unklar, da ebenfalls von einem Einfluss von

Phototropin auf die Expression der Enzyme der Chlorophyllbiosynthese berichtet worden ist (Im *et al.*, 2006).

Plastochinon, ein Zweielektronenüberträger zwischen PSII und Cyt b_6f-Komplex, ist seit längerem als Redoxsignal bekannt. Der Redoxzustand des PQ führt mit Hilfe des Cyt b_6f-Komplexes zu einem Phänomen, welches erstmals 1969 sowohl bei *Chlorella pyrenoidosa* als auch bei *Porphyridium cruentum* beobachtet wurde: der sogenannten *state transition* (Allen, 1992a, b; Bonaventura und Myers, 1969; Murata, 1969). Wie bereits in 1.4 beschrieben, zeichnet sich die äußere Antenne des PSII durch zwei Besonderheiten aus: sie ist in ihrem Durchschnitt variabel und mobil. Die Mobilität ist die Grundlage für jene Kurzzeitantwort, die als *state transition* bezeichnet wird.

Bei Überanregung von PSII kommt es zur Reduktion des PQ-Pools. Eine Folge dieser Reduktion ist die Phosphorylierung von LHCII Untereinheiten der äußeren Antenne und deren Loslösung von der inneren Antenne. Diese phosphorylierten LHCII assoziieren mit stromalen Untereinheiten von PSI. Den Zustand, in dem LHCII mit PSI assoziiert ist, nennt man *state 2*. Wahrscheinlich konstitutiv arbeitende LHCII-Phosphatasen dephosphorylieren LHCII, wodurch es sich vom PSI lösen und zu PSII wandern kann (*state1*). Da durch die Verschiebung von LHCII-Antennenkomplexen die Lichtsammeleffizienz für PSII ab- und gleichzeitig für PSI zunimmt, sinkt der Reduktionslevel des PQ-Pools ab. Dies führt zu einer verstärkten Rückwanderung der LHCII zum PSII. Somit stellt sich eine redoxabhängige Gleichgewichtssituation ein.

Diese Art der Energieumverteilung wurde vor allem in einzelligen Grünalgen beobachtet, so zeigte *Chlamydomonas reinhardtii* eine Antennenverschiebung von bis zu 80 % (Delosme *et al.*, 1996). Im Gegensatz zu *Chlamydomonas* verschieben höhere Pflanzen nur etwa 10 % bis 20 % ihrer Antennenkomplexe (Allen, 1992b). Bereits vor 30 Jahren vermutete man, dass eine an die Thylakoidmembran gebundene Kinase für die Phosphorylierung von LHCII verantwortlich ist. Doch erst 2003 konnte sie von Depège *et al*. in einer *Chlamydomonas*-Mutante identifiziert werden (Depège *et al.*, 2003). Dieses Protein wurde Stt7 getauft, wobei Stt für *state transition, thylakoid* steht (Fleischmann *et al.*, 1999; Kruse *et al.*, 1999). In *A. thaliana* fand man ein orthologes Gen zu Stt7, dessen Produkt LHCII-Untereinheiten phosphorylieren konnte (Bellafiore *et al.*, 2005). Diese Kinase wurde, in Anlehnung an Stt7, STN7 genannt.

Der Redoxzustand des PQ-Pools hat bei länger anhaltendem Ungleichgewicht der Energieverteilung zwischen den Photosystemen auch noch eine Langzeitantwort zur Folge. 1995 konnte dies in der Alge *Dunaliella tertiolecta* gezeigt werden (Escoubas *et al.*, 1995). Es war zu dieser Zeit bekannt, dass Akklimation an verschiedene Lichtverhältnisse Veränderungen in der LHC-Abundanz nach sich ziehen kann und dass diese Akklimationen reversibel sind (Sukenik *et al.*, 1990). Mittels verschiedener Lichtstärken, DCMU und DBMIB wurde gezeigt, dass der Redoxzustand des PQ die nukleäre Transkription eines *Lhc*-Gens beeinflusst und somit PQ die Quelle eines Plastidensignals darstellt oder ein Plastidensignal moduliert. In *Sinapis alba* wurde gezeigt, dass der Redoxzustand des PQ ebenso eine Regulatorfähigkeit auf Ebene der plastidären Transkription besitzt (Pfannschmidt *et al.*, 1999a). Durch die Verwendung von den Inhibitoren DCMU und DBMIB konnten diese Messungen an isolierten Chloroplasten ohne Beisein zytosolischer Photorezeptoren den Einfluss des Redoxzustandes des PQ-Pools auf die Transkriptionsaktivität und die

Transkriptmenge plastidär kodierter Photosynthesegene zeigen. Bei den Untersuchungen von Pfannschmidt *et al*. handelte es sich um das Gen *psbA*, kodierend für D1 – ein Protein des Reaktionszentrums von PSII, und das Operon *psaAB*, welches für die Proteine PsaA und PsaB, die Reaktionszentrumproteine A1 und A2 von PSI, kodiert. Der Redoxzustand des PQ-Pools wurde nicht nur durch Inhibitoren, sondern auch durch unterschiedliche Lichtqualitäten, die bevorzugt PSI oder PSII anregten, moduliert. Die Lichtqualität, die bevorzugt PSII anregt, wurde PSII-Licht genannt und *vice versa* wurde die bevorzugt PSI anregende Lichtquelle PSI-Licht genannt.

Abbildung 1 verdeutlicht die in 1.4.2.1 und 1.4.2.2 vorgestellten redoxkontrollierten Signalwege, wobei in dieser Übersicht die *state transition* weggelassen wurde. In ihr wird deutlich, dass die Expression plastidärer Photosynthesegene sowohl durch perzeptionelle als auch transduktionelle Redoxkontrolle moduliert wird. Ebenso zeigt sich ein Einfluss transduktioneller Signale auf Ebene der Translation von plastidären Genen des Photosyntheseapparates. Für die Vermittlung des perzeptionellen Redoxsignals des Plastochinonpools wird ein Zwei-Komponentensystem als weiterer Signalüberträger vermutet. Diese Vermutung wurde bis heute weder bestätigt noch wurde sie widerlegt. Signale, die durch die Photosynthese erzeugt werden, können ebenfalls die Plastiden verlassen und Effekte auf die Kerngenexpression zeigen. Die Signaltransduktionswege sind auch in diesem Fall noch nicht aufgeklärt.

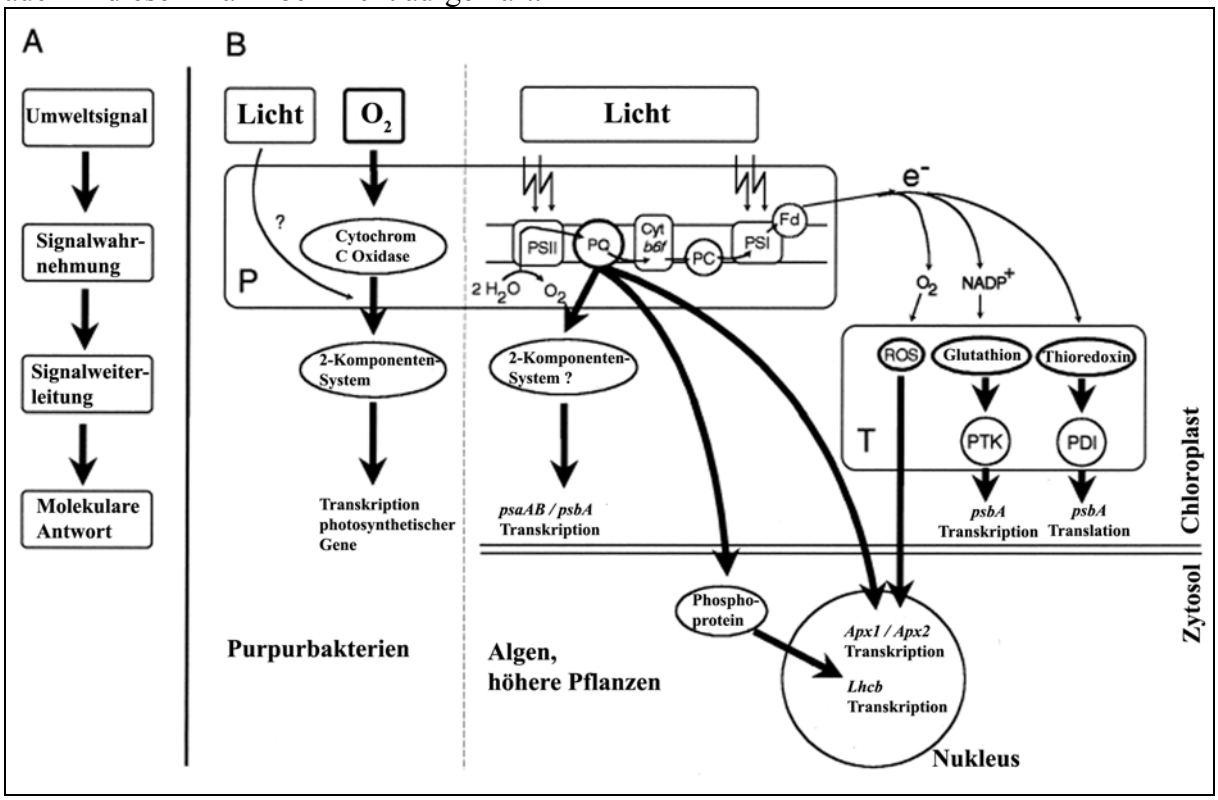

Abb. 1: Schematische Darstellung perzeptioneller Redoxkontrolle in verschiedenen photosynstetischen Organismen. Abb. 1A stellt die Teilschritte von Reizaufnahme bis zur Antwort auf den Reiz dar. Abb. 1B zeigt die jeweils in A dargestellte Unterkategorie an perzeptionellen (P) und transduktionellen (T) Redoxsignal-Prozessen in Purpurbakterien und höheren Pflanzen. PSII: Photosystem II, PQ: Plastochinonpool, Cyt *b6f*: Cytochrom *b6f*-Komplex, PC: Plastocyanin, PSI: Photosystem I, Fd: Ferredoxin, ROS: Reaktive Sauerstoffspezies, PTK: Plastidäre Transkriptionskinase, PDI: Disulfidisomerase ähnliches Enzym RNA-Bindeprotein 60 (RB60). Modifiziert nach Pfannschmidt *et al.*, 2001a.

1.5 Die Messung der photosynthetischen Chlorophyllfluoreszenz als nicht-invasives Werkzeug zur Beurteilung der Photosyntheseeffizienz

Chlorophylle sind Tetrapyrrolringsysteme, welche durch ihre konjugierten Doppelbindungen Licht absorbieren können. Durch die Absorption eines Lichtquants kann ein Übergang des jeweiligen Moleküls in einen angeregten Zustand erfolgen. Eine Relaxation dieses Zustandes kann auf unterschiedlichem Weg erfolgen. Chlorophylle in organischen Lösungsmitteln zeigen bei Belichtung eine konstante Fluoreszenz unabhängig von vorhergehender Probenverdunkelung (Schreiber *et al.*, 1998). Dies ist nicht der Fall bei den Chlorophyllen des Photosyntheseapparates. Bereits 1931 wurde beschrieben, dass die Chlorophyllfluoreszenz von Pflanzen in ihrer Intensität variiert. So konnte beobachtet werden, dass bei Beleuchtung mit UV- oder Blaulicht nach Dunkelakklimation die Chlorophyllfluoreszenz sehr stark ansteigt und dann langsam wieder abfällt. Weiterhin sah man bei Vergiftung des Gewebes mit Blausäure oder bei Temperaturen um den Gefrierpunkt zwar den Anstieg der Chlorophyllfluoreszenz, doch nicht ihre Verminderung (Kautsky und Hirsch, 1931). Dieser Effekt der Chlorophyllfluoreszenzinduktion und -verminderung bei Beleuchtung nach Dunkelakklimation wird heute noch häufig als Kautskyeffekt bezeichnet. Dennoch dauerte es noch einige Dekaden, bis technischer und wissenschaftlicher Fortschritt es ermöglichten, Fluoreszenzanstieg und –abfall genauer zu charakterisieren. Einige der heute verwendeten technischen Verfahren basieren auf dem Prinzip der Puls-Amplituden-Modulation (PAM). Diese ermöglicht es dem Detektionssystem, die durch ein moduliertes Messlicht induzierte Fluoreszenz von Fluoreszenzsignalen zu unterscheiden, die durch Umgebungslicht hervorgerufen werden (Schreiber *et al.*, 1986).

Verschiedene Experimente führten schließlich zu einem Modell, welches die ersten Schritte einer Fluoreszenzinduktion verdunkelter Blätter beschreibt: das *reversible radical pair model* (Schatz *et al.*, 1988). Die ersten Schritte der Ladungstrennung des P_{680} im PSII betreffen drei in PSII gebundene prostetische Gruppen: P_{680} selbst, Phäophytin (Phäo) und das Plastochinon (Q_A). Der Redoxzustand dieser drei Gruppen beeinflusst die Energieverwertung des Photosystemkomplexes und ist für den oben beschriebenen Chlorophyllfluoreszenzanstieg verantwortlich. Man geht davon aus, dass bei höheren Pflanzen der Plastochinonpool in Dunkelheit in einem oxidierten Zustand vorliegt. Ebenso wie der Plastochinonpool ist auch das Q_A des PSII oxidiert. Diesen Zustand des PSII bezeichnet man als „offen“, d.h. von den Antennen eingefangene Photonen, welche zu einer Ladungstrennung im Photosystemzentrum führen, werden mit hoher Wahrscheinlichkeit in den linearen Elektronentransport eingespeist. Den Ausgangszustand des PSII in Dunkelheit kann man als [P_{680} Phäo Q_A] beschreiben. Bei Lichtabsorption geht das System in einen angeregten Zustand über: [P_{680} Phäo Q_A]*, welcher über Fluoreszenz, Wärmeabgabe oder durch die Übertragung eines Elektrons von P_{680} auf Phäophytin (die Bildung des *radical pair*) relaxiert werden kann: [P_{680}^+ Phäo$^-$ Q_A]. Dieser letztgenannte Zustand kann wiederum über verschiedene Wege relaxieren: den photochemischen Weg, der Formung eines Triplettzustandes und der Abgabe von Wärme.

Solange Q_A oxidiert ist, besteht eine hohe Wahrscheinlichkeit für die Weitergabe des Elektrons an Q_A und somit der Verrichtung photosynthetischer Arbeit: [P_{680}^+ Phäo Q_A^-], wodurch die Wahrscheinlichkeit für eine Energieabgabe durch Fluoreszenz sehr gering ist. Falls P_{680}^+ durch den Wasserspaltungsapparat wieder reduziert wurde und es ein weiteres Mal zur Ladungstrennung kommt, Q_A jedoch noch immer reduziert ist, sinkt die

Wahrscheinlichkeit für die Bildung des radikalen Paares durch die elektrostatische Abstoßung negativer Ladungen zwischen $Phäo^-$ und Q_A^-. Gleichzeitig steigt die Wahrscheinlichkeit für eine Relaxation durch Fluoreszenz. Dieser Effekt ist beobachtbar bei dunkelakklimatisierten höheren Pflanzen. Bei Gabe eines schwachen nichtaktinischen Messlichts erhält man die Grundfluoreszenz F_0, welche die Antennenautofluoreszenz zeigt. In diesem Zustand sind fast alle PSII Zentren offen. Bei Gabe eines starken sättigenden Lichtpulses werden alle PSII-Zentren geschlossen, wobei das photochemische *Quenching* gegen Null geht und die Fluoreszenz maximal ist. Diese Fluoreszenzintensität bezeichnet man als maximale Fluoreszenz (F_m) (Abb. 2).

Das System $[P_{680}^+ Phäo^- Q_A]$ kann auch vom Singulett- über *inter-system-crossing* in Tripplett-Zustand übergehen und relaxieren: $[P_{680}^+ Phäo^- Q_A]^3$. Triplettchlorophylle sind reaktiv. Abgegebene Elektronen werden jedoch häufig von Carotinoiden abgefangen, um eine Beschädigungen des Photosyntheseapparates oder der Lipidmembranen durch Radikale zu vermeiden.

Die erwähnte Wärmeabgabe erfolgt strahlungslos und vermindert somit die Fluoreszenzausbeute.

Der oben beschriebene Fluoreszenzanstieg nach Dunkelakklimation ist somit einfach erklärbar, während das Chlorophyllfluoreszenzmuster bei Bestrahlung mit aktinischem Licht der nächsten Minuten durch verschiedene Faktoren beeinflusst wird (Abb. 2).

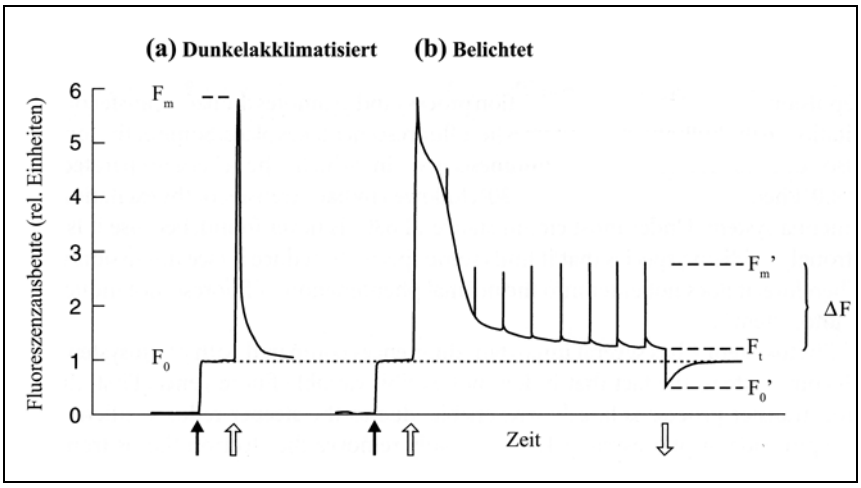

Abb. 2: Schematischer Verlauf der relativen Chlorophyllfluoreszenzausbeute gemessen nach der Sättigungspulsmethode. Auf der Abszisse des Diagramms ist die Zeit dargestellt, während auf der Ordinate die relative Fluoreszenzausbeute dargestellt wird. Abbildung (a) zeigt, dass dunkelakklimatisierte Pflanzen während Bestrahlung mit einem schwachen modulierten Messlicht von etwa 620 nm bei einer Intensität von wenigen Mikroeinstein (der Aktivierungszeitpunkt ist mit einem schwarzen Pfeil markiert) die Grundfluoreszenz F_0 zeigen, welche die Antennenautofluoreszenz widerspiegelt. Durch Gabe eines sättigenden Starklichtpulses (weißer Pfeil) erreicht man eine maximale Fluoreszenzausbeute F_m, bei welcher fast alle PSII-Zentren geschlossen vorliegen. Abbildung (b) zeigt das Abklingen der Chlorophyllfluoreszenz bei kontinuierlicher Beleuchtung. Nach einem anfänglichen schnellen Anstieg sinkt die Chlorophyllfluoreszenz und geht nach einigen Minuten in einen Gleichgewichtszustand (*steady state*) über. Der schwarze Pfeil zeigt die Aktivierung des Messlichts an, während der weiße Pfeil Aktivierung, bzw. Deaktivierung des aktinischen Lichts anzeigt. Fluoreszenzspitzen werden durch Gabe einzelner Starklichtpulse erzeugt. F_m' ist somit die Maximalfluoreszenz zu einem gegebenen Zeitpunkt der Beleuchtungsphase. F_t ist die Chlorophyllfluoreszenzintensität bei aktinischem Licht zu einem gegebenen Zeitpunkt. Nach Deaktivierung des aktinischen Lichts erhält man die Grundfluoreszenz F_0'des lichtakklimatisierten Zustands. Modifiziert nach Schreiber *et al.*, 1998.

Es zeigte sich, dass die bei Raumtemperatur beobachtbare variable Chlorophyllfluoreszenz von *Plantae* hauptsächlich von PSII stammt. PSI trägt nicht zur variablen Chlorophyllfluoreszenz bei. Der Grund dafür ist noch nicht genau geklärt (Knüppers und Häder, 1999), doch scheint die Ursache dafür darin zu liegen, dass der Elektronentransfer im PSI irreversibel ist. Dieser Vermutung liegen mehrere Fakten zu Grunde. Der Redoxgradient zwischen P_{700} und seinem Elektronenakzeptor ist größer als derjenige bei P_{680} und Phäophytin. Hinzu kommt, dass der P_{700}-Elektronenakzeptor sterisch weiter von P_{700} entfernt ist, wodurch der elektrostatische Effekt zwischen gegensätzlichen Ladungen gemindert wird und keine Bildung eines *radical pair* stattfindet. Weiterhin wurde beobachtet, dass oxidiertes P_{700} (P_{700}^{+}) ebenso gut von der PSI-Antenne angeregt werden kann, wie P_{700}: $P_{700}^{+} \rightarrow P_{700}^{+*}$, wodurch die Wahrscheinlichkeit auf eine Energieabgabe durch Fluoreszenz stark verringert ist (Blankenship, 2002).

1.5.1 Messung und Interpretation wichtiger Chlorophyllfluoreszenzparameter

In der Vergangenheit wurde eine Vielzahl von Parametern diskutiert, welche in die Chlorophyllfluoreszenzvariabilität einfließen. Diese Parameter werden in ihrer Gesamtheit als Fluoreszenz-*Quenching*-Parameter bezeichnet. Vereinfacht kann man sagen, dass die Energie der von PSII absorbierten Strahlung in Photochemie, Fluoreszenz und strahlungslose Dissipation umgewandelt wird. Die Vorgänge der strahlungslosen Dissipation vereinen verschiedene Vorgänge, welchen verschiedene *Quenching*-Parameter zugeordnet werden, wobei für eine Quantifizierung vor allem das nichtphotochemische Quenching (NPQ) verwendet wird, welches die Hitzedissipation des Systems beschreibt (Maxwell und Johnson, 2000). Der Anteil eines jeweiligen Prozesses im Verhältnis zur Summe aller Prozesse wird als Ausbeute bezeichnet.

In gängiger Laborpraxis werden die Parameter häufig mit einem Puls-Amplituden-Modulations (PAM)-Fluorometer bestimmt. Das PAM-Fluorometer verwendet das in Abbildung 2 dargestellte Messprinzip. Die zentrale Annahme für die Quantifizierung der Chlorophyllfluoreszenzparameter lautet: das Verhältnis der Geschwindigkeitskonstanten für Fluoreszenzemission und strahlungsloser Dissipation bleibt während eines Sättigungspulses konstant.

Der Prozess der Photochemie des PSII wird in Standardmessungen häufig über den Genty-Parameter beschrieben (Genty *et al.*, 1989). Dieser beschreibt die maximale und effektive Quantenausbeute Φ_{II} und leitet sich aus dem Fakt der Einheit der drei möglichen Ausbeuten für Photochemie (Φ_P), Fluoreszenz (Φ_F), strahlungslose Dissipation (Φ_D):

$$\text{Gl. 1.5.1a: } 1 = \Phi_P + \Phi_F + \Phi_D$$

und der oben getroffenen Annahme her:

$$\text{Gl. 1.5.1b: } \frac{\Phi_{Fm}}{\Phi_{Dm}} = \frac{\Phi_F}{\Phi_D}$$

wobei während des Sättigungspulses, durch die Schließung aller PSII-Zentren, die photochemische Ausbeute gegen Null geht. Dagegen erreichen die Ausbeuten für Fluoreszenz und strahlungslose Übergänge ein Maximum (Φ_{Fm}, Φ_{Dm}).

Gl. 1.5.1c: $1 = \Phi_{Fm} + \Phi_{Dm}$

Durch Umformung von Gleichung 1.6.1c und Substitution in Gleichung 1.6.1b erhält man eine Beschreibung der Ausbeute strahlungsloser Dissipation mittels der Fluoreszenzausbeute:

Gl. 1.5.1d: $\Phi_D = \frac{\Phi_F}{\Phi_{Fm}} - \Phi_F$

Durch Einsatz dieses Terms für Φ in Gleichung 1.6.1a ist es nun möglich die photochemische Ausbeute allein durch die Fluoreszenzausbeute zu charakterisieren:

Gl. 1.5.1e: $\Phi_P = \frac{\Phi_{Fm} - \Phi_F}{\Phi_{Fm}}$

Durch diese Herleitung ist es möglich, die maximale Quantenausbeute von PSII nach Dunkelakklimation zu bestimmen:

Gl. 1.5.1f: $\Phi_{II\,m} = \frac{F_m - F_0}{F_m} = \frac{F_v}{F_m}$

Weiterhin lässt sich die effektive Quantenausbeute von PSII während der Bestrahlung mit aktinischem Licht wie folgt berechnen:

Gl. 1.5.1g: $\Phi_{II} = \frac{F_m' - F_t}{F_m'} = \frac{\Delta F}{F_m'}$

Während der Genty-Parameter den Anteil der absorbierten Energie, der für Photochemie genutzt wird, widerspiegelt, kann über das photochemische *Quenching* qP der Anteil offener PSII-Zentren abgeschätzt werden:

Gl. 1.5.1h: $qP = \frac{F_m' - F_t}{F_m' - F_0'}$

Da qP einen Wert zwischen Null und Eins einnimmt, zeigt 1-qP den Anteil an geschlossenen PSII-Zentren und somit PSII-Zentren mit reduziertem Plastochinon Q_A an (Maxwell und Johnson, 2000).

Wärmeabgabe zählt zu den strahlungslosen Dissipationsformen, da die Thermostrahlung nicht als Fluoreszenz messbar ist. Sie wird am besten über den nicht-photochemischen *Quenching*-Parameter (NPQ) beschrieben:

Gl. 1.5.1i: $NPQ = \frac{F_m - F_m'}{F_m'}$

Ein Äquivalent dieses Parameters ist das qN (van Kooten und Snel, 1990). Dieser Parameter ist jedoch bei einem starken Anstieg des nicht-photochemischen *Quenchings* limitierend, da seine Skalierung zwischen Null und Eins liegt, wodurch er bei hohen Messwerten unsensitiv wird. Der Vollständigkeit halber seien noch drei weitere *Quenching*-Parameter aufgeführt, welche man mittels eines entsprechenden Messaufbaus und durch ihre unterschiedlichen Relaxationskinetiken unterscheiden kann. Alle drei Parameter beschreiben strahlungslose Dissipationsprozesse. Das Hochenergie-*Quenching*, qE, findet bei starkem Lichteinfall in Gegenwart eines hohen Protonengradienten über der Thylakoidmembran statt. Es schützt die Blätter vor Starklichtschäden durch Formation von Zeaxanthin (Adams *et al.*,

1999). Weiterhin trägt die bereits erläuterte *state transition*, bekannt als qT, zum *Quenching* bei (Walters und Horton, 1991). Der dritte *Quenching*-Prozess, qI, entsteht durch die Photoinhibition langbestrahlter PSII-Zentren.

1.5.2 Lichtqualitätsakklimation führt zu unterschiedlichen relativen *steady state*-Fluoreszenzintensitäten

Durch anhaltende Unterschiede in der einstrahlenden Lichtqualität reagieren Pflanzen mit Akklimationsreaktionen, die Einfluss auf Genexpression, Biochemie, strukturelle Veränderungen und Entwicklungsprozesse haben (Deng *et al.*, 1989; Pfannschmidt *et al.*, 1999b). Für *Sinapis alba* konnte gezeigt werden, dass nach dem Eintreten der Gleichgewichtschlorophyllfluoreszenz (*steady state*-Chlorophyllfluoreszenz), starke Unterschiede in der relativen Chlorophyllfluoreszintensität existierten (Pfannschmidt *et al.*, 1999b). Es wurde vermutet, dass diese Unterschiede der Fluoreszenzintensität auf der Anpassung der Photosystemstöchiometrie an die Lichtqualitäten und/oder durch eine Veränderung der Antennen basieren. Die Daten lieferten jedoch keine direkt quantifizierbaren Fluoreszenzparameter, da sowohl F_0 als auch F_m fehlten.

Durch Messungen mit einem PAM-Fluorometer war es möglich diesen physiologischen Parameter semiquantitativ zu erfassen:

Gl 1.5.2a: $$\frac{F_s}{F_m} = \frac{F_t - F_0'}{F_m}$$

F_s/F_m beschreibt die Gleichgewichts-Chlorophyllfluoreszenz in Relation zur maximal möglichen Fluoreszenz und unabhängig zur Grundfluoreszenz nach Belichtung (F_0'). Da die Grundfluoreszenz ein Parameter für die Autofluoreszenz der Antennen ist, kann man in erster Näherung behaupten, dass unterschiedliche F_s/F_m-Werte bei konstantem NPQ eine Veränderung der Photosystemstöchiometrie widerspiegeln. Die Abbildung 3 zeigt schematisch die relativen Fluoreszenzkinetiken von Pflanzen, die an PSI- oder PSII-Licht angepasst sind (Pfannschmidt *et al.*, 2001a).

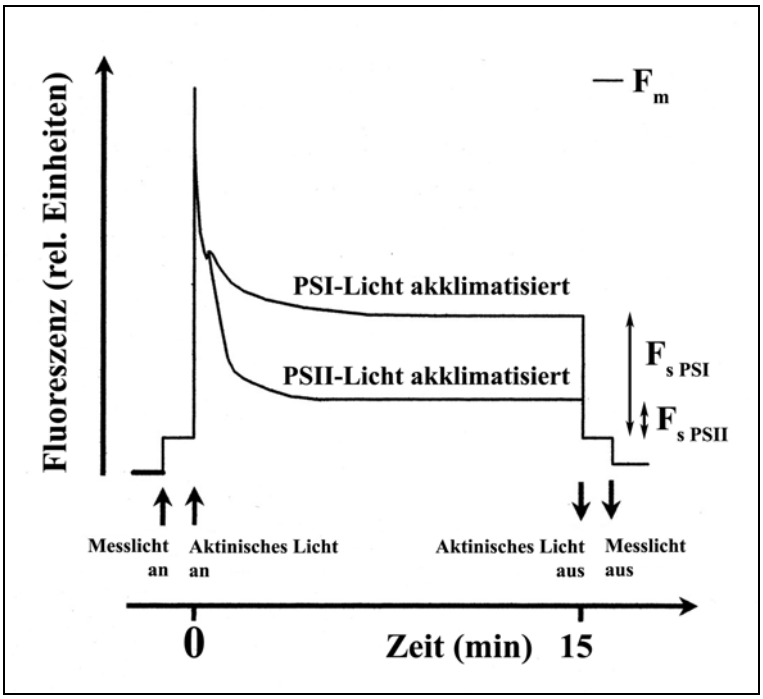

Abb. 3: Schematische Darstellung der Chlorophyll-Fluoreszenz-Kinetik von Tabakpflanzen, die an PSI- oder PSII-Licht akklimatisiert wurden. F_m: maximale Fluoreszenz, F_{sPSI}: F_t-F_0' einer PSI-Licht akklimatisierten Pflanze, F_{sPSII}: F_t-F_0' einer PSII-Licht akklimatisierten Pflanze. Die Abbildung wurde nach Pfannschmidt *et al.*, 2001b, modifiziert.

1.6 Fragestellungen

Welche physiologischen Parameter unterscheiden sich nach Akklimation an PSI/PSII-Licht in *A. thaliana* und ist sie als Modellsystem für die Untersuchung von Lichtakklimation geeignet?

Gibt es Einflüsse des photosynthetischen Elektronentransports auf nukleäre und plastidäre Genexpression in *A. thaliana* und wenn ja, welche sind dies?

Verändert sich die Proteinkomposition der Thylakoidmembran während einer Lichtqualitätslangzeitakklimation in *A. thaliana*?

Ist es möglich mittels eines Ausleseverfahrens Mutanten mit einer gestörten Langzeitakklimation an Lichtqualitätsverhältnisse von *A. thaliana* zu identifizieren?

Wie könnten Redoxsignale auf die Genexpressionsebene übertragen werden?

2 Manuskriptübersicht

I)
Wagner, R., Fey, V., Borgstädt, R., Kruse, O. und Pfannschmidt, T. **(2004)**. "Screening for Arabidopsis thaliana mutants deficient in acclimatory long-term response to varying light qualities using chlorophyll fluorescence imaging." In: 13th International Congress of Photosynthesis. Bruce, D. Montréal, Allen Press**:** 693-695.

In dieser Arbeit wurden die Unterschiede in der Anpassung von *A. thaliana* an PSI- und PSII-Licht mittels eines zweidimensional auflösenden Verfahrens zur Messung der Chlorophyllfluoreszenz dargestellt. Basierend auf der ermittelten Wildtypantwort wurde ein *Screening* Verfahren entwickelt mittels dessen Pflanzen selektiert werden können, die keine Veränderungen der Gleichgewichtschlorophyllfluoreszenz nach Abschluss der Lichtqualitätsakklimation zeigen. An einer mutagenisierten Saatgutpopulation von *A. thaliana* wurden bei der Etablierung des Verfahrens Indizien dafür gefunden das möglicherweise zwei verschiedene intrazelluläre Informationskanäle genutzt werden, um auf unterschiedliche Lichtqualitäten zu reagieren.
Raik Wagner entwickelte die *Screening*-Methode anhand des von Vidal Fey, Raik Wagner und Thomas Pfannschmidt etablierten Fluoreszenzparameters F_s/F_m durch Verwendung einer PAM101-PDA100 Kombinationseinheit. Raik Wagner führte Pflanzenanzucht, Messung und statistische Prüfung der Messergebnisse durch.

II)
Fey, V., **Wagner, R.**, Bräutigam, K., Wirtz, M., Hell, R., Dietzmann, A., Leister, D., Oelmüller, R., and Pfannschmidt, T. **(2005)**. "Retrograde plastid redox signals in the expression of nuclear genes for chloroplast proteins of *Arabidopsis thaliana*." Journal of Biological Chemistry 280(7): 5318-5328.

Dieser Artikel zeigt auf, dass bei Anpassung an verschiedene Lichtqualitäten die Transkription von plastidären (nachgewiesen mit einem von Vidal Fey angepassten *Primer Extension*-Verfahren) und nukleären Genen (gezeigt mittels Array-Technologie) in *A. thaliana* betroffen sind. Weiterhin wurde gezeigt, dass die Veränderung der Photosystemstöchiometrie bei Lichtqualitätsverschiebung in *A. thaliana* vorwiegend auf einer Mengenveränderung des Photosystem I in Bezug auf die Gesamtproteinkonzentration beruht. Durch Messung des Gehaltes und Redoxzustandes von Glutathion konnte nachgewiesen werden, dass die verwendeten Lichtqualitäten auf dieses Antioxidant-System keinen Einfluss ausüben und damit eine Beteiligung reaktiver Sauerstoffspezies an dieser Reaktion unwahrscheinlich ist.
Raik Wagner führte die *Western*-Analyse der Zentrumproteine der Photosysteme I und II und von einzelnen Untereinheiten der Antennenkomplexe durch. Ebenso war er an der Pigmentbestimmung beteiligt. Raik Wagner, Katharina Bräutigam und Thomas Pfannschmidt sortierten und interpretierten die Expressionsdaten aus den Array-Analysen. Das Material für die Glutathionbestimmung wurde von Raik Wagner vorbereitet.

III)
Fey, V., **Wagner, R.**, Bräutigam, K., Pfannschmidt, T. **(2005)**. "Photosynthetic redox control of nuclear gene expression." Journal of Experimental Botany 56(416): 1491-1498.

In diesem Artikel werden die Erkenntnisse der letzten Jahre zum Gebiet der photosynthetischen Expressionsregulation nukleärer Gene zusammengefasst. Dabei wird besonderes Augenmerk auf die Regulation solcher Genprodukte gelegt, die in die Plastiden importiert werden müssen. Es wird diskutiert, welche photosynthetischen Parameter bereits dafür bekannt sind, nukleäre Genexpression zu verändern. In einem Modell werden Plastidensignale und Signale zytosolischer Photorezeptoren dargestellt und es wird diskutiert, wie diese interagieren können.
Vidal Fey, Raik Wagner, Katharina Bräutigam und Thomas Pfannschmidt haben diesen Artikel verfasst. Die Diskussion des Kenntnisstandes, Erarbeitung theoretischer Modelle und Korrekturlesung erfolgte durch alle beteiligten Autoren.

IV)
Bonardi, V., Pesaresi, P., Becker, T., Schleiff, E., **Wagner, R.**, Pfannschmidt, T., Jahns, P., Leister, D. **(2005)**. "Photosystem II core phosphorylation and photosynthetic acclimation require two different protein kinases." Nature 437(7062): 1179-1182.

In dieser Arbeit wird gezeigt, dass die plastidär lokalisierte Kinase STN8 in *Arabidopsis* für die Phosphorylierung von Untereinheiten des Photosystems II verantwortlich ist und dass diese Phosphorylierung nicht geschwindigkeitsbestimmend für Abbau und Neusynthese von D1 ist. Des Weiteren wurden in dieser Arbeit Arabidopsis *knock-out*-Linien für die Proteine von STN7, STN8 und die Doppelmutante *stn7/stn8* mittels des im Manuskript I entwickelten und optimierten Ausleseverfahrens untersucht. Im Gegensatz zum Wildtyp und der *stn8-knock-out*-Linie zeigten weder die *stn7-knock-out*-Linie noch die Doppelmutante *stn7/stn8* eine Veränderung von Chlorophyllfluoreszenzparametern und Pigmentzusammensetzung. Dadurch war ersichtlich, dass die STN7-Kinase nicht nur für die Kurzzeitantwort, sondern auch für die Langzeitantwort essentiell ist.
Die Messungen zur Akklimation an veränderte Lichtqualitäten, d.h. Photosyntheseparameter und Pigmentbestimmungen, wurden von Raik Wagner und Thomas Pfannschmidt durchgeführt, ausgewertet und interpretiert.

V)
Wagner, R., Bräutigam, K., Pfannschmidt, T. **(2006)**. "Die Photosynthese – Ein Umweltsensor, der Gene reguliert." Bioforum 3: 48-50.

In diesem kurzen deutschen Übersichtsartikel wurden die aktuellen Entwicklungen für ein breites Publikum zusammengefasst, nämlich dass der Prozess der Photosynthese gleichermaßen chemische Energie als auch Informationen an die Pflanze liefert. Diese Informationen scheinen von der Pflanze interpretierbar zu sein, da diese dann sinnvoll auf Umweltveränderungen reagiert, in dem sie ihre Proteinausstattung modifiziert und erneuert.

Am Verfassen des Artikels waren alle drei Autoren beteiligt. Die Messungen von einfallender visueller Strahlung unter natürlichen Bedingungen wurden von Katharina Bräutigam und Raik Wagner durchgeführt. Abbildung 1 wurde von Katharina Bräutigam, Abbildung 2 und Abbildung 3 wurden von Raik Wagner und Thomas Pfannschmidt erstellt.

VI)
Wagner, R. und Pfannschmidt, T. **(2006)**. "Eukaryotic transcription factors in plastids - Bioinformatic assessment and implications for the evolution of gene expression machineries in plants." Gene 381: 62-70.

In dieser Veröffentlichung wird erstmals geschätzt, dass zwischen 50 und 100 eukaryotische Transkriptionsfaktoren in Plastiden importiert werden. Weiterhin stellen wir ein Modell vor, welches die Möglichkeit skizziert, wie die Substitution ursprünglich in Plastiden vorhandener Signalwege und Transkriptionsfaktoren erfolgt sein könnte.
Bioinformatische Analysen von Transit- und Kernlokalisationssequenzen, Datenbank-extraktion, Suche von putativen plastidären *cis*-Elementen für eukaryotische Transkriptions-faktoren und Überprüfung der Annotation wurden von Raik Wagner durchgeführt. Dateninterpretation, Literatursuche und die Entwicklung des Evolutionsmodells wurden von Raik Wagner und Thomas Pfannschmidt durchgeführt.

VII)
Wagner, R., Fischer, W., Leister, D. and Pfannschmidt, T. **(2006)**. "The role of long-term light quality acclimation in photosynthetic performance of *Arabidopsis thaliana*." Manuskript in Vorbereitung für Plant Physiology.

In diesem Manuskript werden verschiedene physiologische Leistungen von *A. thaliana* nach Akklimation an PSI- und PSII-Licht wiedergegeben. Mutanten mit Defizienzen in der Kurz- und/oder der Langzeitantwort werden zum Vergleich herangezogen, um die Bedeutung photosynthetischer Lichtqualitäts-Akklimation für die einzelne Pflanze zu zeigen.

4 Manuskripte

I

Wagner, R., Fey, V., Borgstädt, R., Kruse, O. und Pfannschmidt, T. **(2004)**. "Screening for Arabidopsis thaliana mutants deficient in acclimatory long-term response to varying light qualities using chlorophyll fluorescence imaging." In: 13th International Congress of Photosynthesis. Bruce, D. Montréal, Allen Press**:** 693-695.

In dieser Arbeit wurden die Unterschiede in der Anpassung von *A. thaliana* an PSI- und PSII-Licht mittels eines zweidimensional auflösenden Verfahrens zur Messung der Chlorophyllfluoreszenz dargestellt. Basierend auf der ermittelten Wildtypantwort wurde ein *Screening* Verfahren entwickelt mittels dessen Pflanzen selektiert werden können, die keine Veränderungen der Gleichgewichtschlorophyllfluoreszenz nach Abschluss der Lichtqualitätsakklimation zeigen. An einer mutagenisierten Saatgutpopulation von *A. thaliana* wurden bei der Etablierung des Verfahrens Indizien dafür gefunden das möglicherweise zwei verschiedene intrazelluläre Informationskanäle genutzt werden, um auf unterschiedliche Lichtqualitäten zu reagieren.

Table 2: Differential gene expression of two gene clusters in Δ*isiA* Compared to wild type *Synechocystis* sp. PCC 6803

Gene	Putative Function	Fold Change[b] WT	Δ*isiA*
Control (No H_2O_2)			
sll1693	SAM–dependent methyl transferase	1.1[c]	2.1
sll1694	PilA1, GspG	1.1	2.4
sll1695	PilA2, GspH	1.1	3.7
sll1696	hypothetical	1.1	5.2
slr1456	PilA4, GspG	1.8	2.0
slr0079	PilB2, GspE2 (ATPase)	1.0	3.5
+1.5 mM H_2O_2			
sll1158	Flavoprotein (I TMS)[a]	2.1	3.2
sll1159	peroxiredoxin	3.2	8.4
sll1160	hypothetical (I TMS)	5.5	7.2
sll1161	Adenyl/Guanyl cyclase	3.3	7.0

[a] TMS, transmembrane segment.
[b] Fold change computed as described (Singh et al 2003). A positive fold change means that transcription was enhanced in the Δ*isiA* mutant.
[c] Average from 6 separate experiments.

This may be significant since this was a suggested function for IsiA (Burnap et al 1993), although no direct evidence has been obtained to test this hypothesis. It is important to note that the results of He and Vermaas (1999) were obtained in a ΔPSI/chlL strain and that our results were obtained in Δ*isiA*. Therefore, the involvement of Sll1694/Sll1695 with Chl transfer among pigment-protein complexes might be more important or more apparent under stress conditions, especially where major Chl-protein complexes are missing. Interestingly, Sll1693 strongly resembles a methyl transferase and that pilins (or adhesions) are typically methylated at an N-terminal M or F residue. In addition, both Sll1694 and Sll1695 have an amino acid structure that is consistent with localization to the cytoplasmic membrane or the periplasm. Thus, we postulate that Sll1695, and possibly Sll1694 under certain conditions, may be involved in the biogenesis of various nascent Chl-protein complexes. This process may involve the cytoplasmic membrane and may facilitate a transient interaction between the two membrane systems.

What Happens to Δ*isiA* on Peroxide Addition? If IsiA is important under stress conditions, why is Δ*isiA* more resistant to peroxide damage? As shown in Table 2, the cluster of genes sll1158 to sll1161 is induced by peroxide in wild type, but to an even greater extent in the Δ*isiA* mutant. The key protein in this cluster may be the peroxiredoxin (Sll1159), a protein involved in the detoxification of peroxide. This adaptation by Δ*isiA* may very well represent a key reason why it is more resistant to damage by peroxide. It will be important to study the proteins in this region in much greater detail. The peroxiredoxin has not been characterized, but the strong induction under these and other conditions demonstrate the importance of these proteins for the survival under peroxide stress.

In conclusion, microarray analysis of differential gene expression in a Δ*isiA* mutant, in the presence and absence of peroxide, has highlighted a few major gene clusters. In the absence of peroxide, a set of pilins-like or GSP-like proteins (in other bacteria) are produced in substantial quantity. These proteins may partially compensate for the loss of IsiA in the transfer of Chl from one pigment protein complex to another. Furthermore, this finding might implicate IsiA in membrane biogenesis under many types of stress conditions. In the presence of peroxide, a gene cluster containing a peroxiredoxin is induced and this may be one enzyme that helps to provide Δ*isiA* with greater resistance to peroxide damage.

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SCREENING FOR *ARABIDOPSIS THALIANA* MUTANTS DEFICIENT IN ACCLIMATORY LONG-TERM RESPONSE TO VARYING LIGHT QUALITIES USING CHLOROPHYLL FLUORESCENCE IMAGING

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INTRODUCTION

Photosystem stoichiometry adjustment is an important acclimatory response to excitation imbalances between photosystem I and II which often result from natural occuring light quality gradients. Typically, the photosystem ratio is changed in favour of the rate-limiting photosystem (Melis 1991, Fujita 1997, Allen & Pfannschmidt 2000) which improves excitation energy distribution and photosynthetic efficiency (Chow et al 1990, Allen 1992). Changes in photosystem stoichiometry require co-ordinated changes in the expression of

photosynthesis genes that are encoded both in the nucleus and the chloroplast genome (Race et al 1999, Rodermel 2001, Pfannschmidt 2003). Photosystem stoichiometry adjustment can be experimentally induced by illumination with artificial light sources that differentially excite PSII (PSII-light) or PSI (PSI-light). In higher plants grown under PSII-light the PSII/PSI ratio decreases while it increases under PSI-light (Glick et al 1986, Chow et al 1990, Kim et al 1993, Pfannschmidt et al 1999). This response is fully reversible (Kim et al 1993) and is based on changes in the expression of plastid (Glick et al 1986, Deng et al 1989, Pfannschmidt et al 1999a, b, Tullberg et al 2000) and nuclear genes (Pfannschmidt et al 2001, Sherameti et al 2002) that are controlled by the changes in the redox state of electron transport components (e.g. the plastoquinone pool). The underlying signal transduction pathways and their components are mainly unknown. The target of our approach was to establish a screen for *Arabidopsis* mutants that have defects in their acclimatory long-term response (LTR) to the PSI- and PSII-light sources. Such a forward genetic approach would allow to identify components of regulatory pathways in chloroplasts and the nucleo-cytosolic compartment. Using chlorophyll fluorescence video imaging we demonstrate that differences in chlorophyll fluorescence between PSI- and PSII-light acclimated *Arabidopsis* seedlings can be used as screening parameter to identify individuals with a changed or missing LTR.

MATERIALS AND METHODS

Plant material and growth conditions. Wild-type and mutant seeds (M_2 population after fast neutron bombardment (55Gy) (Lehle Seeds, USA)) of *Arabidopsis thaliana* (Columbia) were surface-sterilised, germinated (after 2 days at 4 °C) and grown on full MS medium containing 2% (w/v) sucrose. All plants were initially grown for 10 days under white light (~30 μE). The LTR was induced by continuous illumination of seedlings with PSI- or PSII-light as indicated. The light sources were described earlier in detail (Pfannschmidt et al 2001b). Pre-selected LTR-mutants from the first screening step were grown to full rosette stage on soil at a temperature of 20–22 °C and 60–80% humidity under a short-day light regime (8 hours light/ 16 hours dark) until the second LTR screening step was performed.

Determination of F_s/F_m values. F_s/F_m values of PSI- and PSII-light acclimated seedlings were recorded by chlorophyll fluorescence video imaging using a Fluorcam 700 MF (Photon System Instruments, Brno, Czech Republic) and the following programme: 10 min dark-adaptation, 3 s measuring F_0, strong light pulse of 1.6 s to determine F_m, actinic light for 10 min to determine F_t, dark relaxation of 2 min for F_0'. The $(F_t\text{-}F_0')/F_m = F_s/F_m$ value was calculated for each individual. The distribution of F_s/F_m-values over all plants was analysed and the standard deviation calculated to define the thresholds for lacking LTRs. Plants with a F_s/F_m-value beyond these

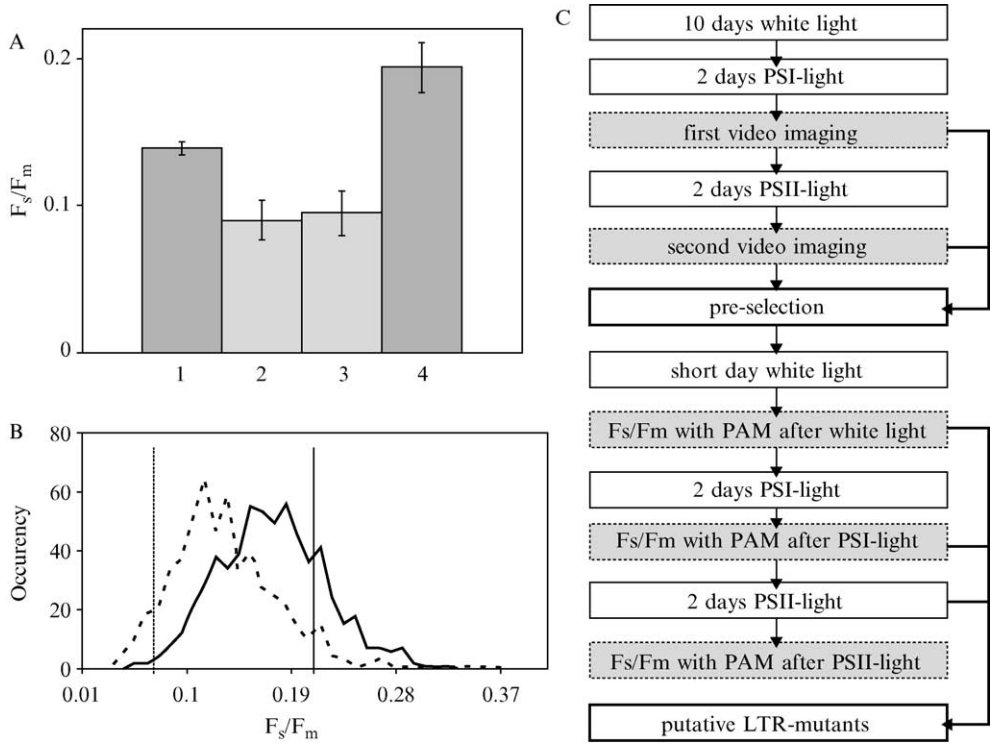

Figure 1: F_s/F_m values in PSI- or PSII-light acclimated *Arabidopsis* seedlings and its use as screening parameter. (A) PAM determination of F_s/F_m from 5 plants each in 3 independent experiments. All plants were grown first for 10 days in white light before the acclimation reactions were induced. Lane 1: 2 days in PSI-light, lane 2: 2 days in PSI-light followed by 4 days in PSII-light, lane 3: 2 days in PSII-light, lane 4: 2 days in PSII-light followed by 4 days in PSI-light. (B) Difference in F_s/F_m of PSI- and PSII-light acclimated mutagenised *Arabidopsis* seedlings. Plants were grown as described followed by F_s/F_m determination with video imaging. Graphs show the distribution of F_s/F_m values of around 600 seedlings (solid line: PSI-light acclimation; broken line: PSII-light acclimation). Thresholds defining putative mutants are indicated by perpendicular lines. (C) Screening strategy for LTR mutants.

thresholds were regarded as putative mutants. In the second screening step the F_s/F_m values of the selected putative mutant were determined in three parallels with a PAM101/PDA100 fluorometer (Walz, Effeltrich, Germany) as described earlier (Pfannschmidt et al 2001).

Chlorophyll determination. Spectroscopic determination of Chl *a* and *b* was performed after extraction of N_2–ground material with 80% buffered acetone according to Porra et al (1989).

RESULTS AND DISCUSSION

White light-grown *Arabidopsis* wild-type seedlings were acclimated to PSI- and PSII-light (PSI- and PSII-plants, respectively) and chlorophyll fluorescence was determined with a PAM101 fluorometer. PSI-plants exhibited a higher F_s/F_m value than PSII-plants. If PSI-plants were acclimated to PSII-light the F_s/F_m value declined while it increased when PSII-plants were acclimated to PSI-light (Fig. 1A). This coincides with earlier observations and demonstrates the reversibility of this acclimatory response (Pfannschmidt et al 2001, Sherameti et al 2002). The kinetic of the LTR was followed by determination of Chl *a/b* and F_s/F_m at different time points after LTR induction. In *Arabidopsis* (Col) the LTR took place to 70–80% within 2 days and was completed after 5–7 days (data not shown). The establishment of a screening protocol for acclimation mutants basing on these changes in F_s/F_m was inspired from screens for state transition mutants in which changes in chlorophyll fluorescence of similar extent were used (Borgstädt and Kruse 2001). To screen chlorophyll fluorescence from many seedlings we used a video imaging camera working with pulse-amplitude modulation allowing a simultaneous F_s/F_m-determination of several individuals. Mutagenised *Arabidopsis* seeds were grown for 10 days under white light followed by 2 days in PSI-light and further 2 days in PSII-light. After each acclimation step chlorophyll fluorescence of the seedlings was determined. A maximum of 25 seedlings per plate could be analysed. The small seedling number prohibited an overlay of leaves due to seedling growth which would interfere with exact F_s/F_m determinations of the individuals. Plants acclimated to PSI-light showed an average F_s/F_m-value of 0.163 (standard deviation (SD): 0.046) while they exhibited an average F_s/F_m-value of 0.123 (SD: 0.047) after the following acclimation to PSII-light. Both averages were significant different from each other as determined by a student t-test of associated samples. The upper border of SD of PSI-plants was set as threshold for a lacking LTR to PSII-light, i.e. PSII-plants exhibiting a F_s/F_m value above the threshold were regarded as individuals lacking the typical response. The lower border of SD of PSII-plants was defined as threshold for plants lacking a LTR to PSI-light i.e. PSI-plants exhibiting a F_s/F_m-value below this threshold were regarded as individuals lacking a proper response (Fig. 1B). The complete screening strategy is shown in Fig. 1C.

Plants meeting these conditions were selected, grown up to full-rosette stage and F_s/F_m values were determined again after a second acclimation to PSI- and PSII-light with a PAM101/PDA100. This two-step strategy was succesful in identifying two different groups of mutants (Fig. 2). One group showed no reaction to the oxidation signal by PSI-light, i.e. it exhibited no increase in the F_s/F_m value while a second group revealed such an increase, however did not show a reaction to the reduction signal by PSII-light since no decrease in the F_s/F_m value could be observed. We regard the groups as putative LTR mutants named LTRI (for the first group lacking the response to PSI-light) and LTRII (for the second group lacking the response to PSII-light). The existence of two groups suggest that the LTR involves more than one regulatory pathway for acclimation. All isolated plants grow autotrophically on soil and none of them shows a phenotype that differs from wild-type under standard white light (Fig. 2) as expected for acclimation mutants.

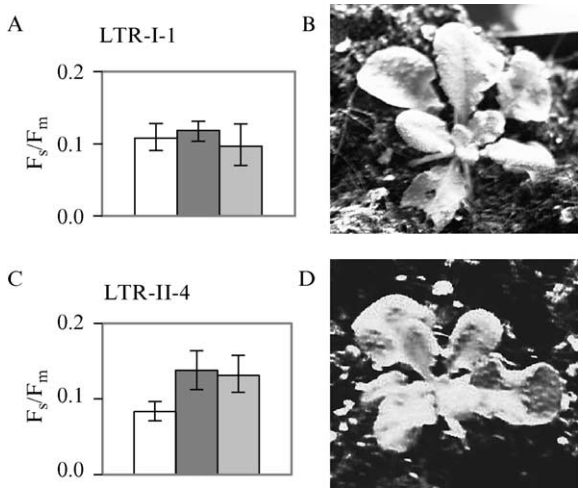

Figure 2: Classes of putative LTR mutants. (A, C) F_s/F_m values of representative plants after several weeks white light (white bar), after 2 days PSI-light (dark-grey bar) and after further 2 days PSII-light (grey bar). (B, D) Phenotypes of LTR-I-1 and LTR-II-4 mutants after 3 weeks in white light.

The screening method presented here therefore is a useful tool to identify mutants with defects in regulatory components of photosynthetic acclimation to varying light qualities and might be of importance also for identifying new regulatory components of photosynthesis in general.

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IDENTIFICATION OF ORFS WHOSE EXPRESSION LEVELS ARE REGULATED BY A SMALL TRANSCRIPTIONAL REGULATOR, SSL0564, IN A CYANOBACTERIUM *SYNECHOCYSTIS* SP. PCC 6803

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Keywords: Cyanobacteria, DNA microarray, GerE, LuxR family, Transcriptional regulator

INTRODUCTION

The majority of bacterial transcriptional regulators use a helix-turn-helix (HTH) motif to bind DNA. In a cyanobacterium, *Synechocystis* sp. PCC 6803, more than 50 transcriptional regulators with a HTH motif are encoded in the genome (Kaneko et al 1996). Most of these transcriptional regulators consist of two domains: a DNA-binding domain containing a HTH motif and a receiver domain activated by its phosphorylation or binding of effector molecules. However, there exists a small transcriptional regulator, Ssl0564, consisting solely of a DNA-binding domain characteristic to LuxR subfamily of transcriptional regulators. Similar small LuxR-type regulators, about 100 amino acid residues in length, are found in wide range of bacteria such as proteobacteria, firmicutes and cyanobacteria (http://smart.embl-heidelberg.de/). Among them, only GerE, a transcriptional regulator of sporulation-specific genes in *Bacillus subtilis*, has been extensively characterized (Cutting et al 1989, Ichikawa et al 1999, Ducros et al 2001). We noticed that all the cyanobacterial species whose genomic sequence has been determined possess at least one small LuxR-type regulator. These cyanobacterial regulators were highly conserved and contained three cysteine residues at their C-termini. When the phylogenetic tree of small LuxR-type regulators was constructed, these cyanobacterial regulators formed a discrete clade. Since C-terminal cysteine residues do not exist in small LuxR-type regulators of other bacterial species, cyanobacterial regulators seem to be involved in regulatory mechanism characteristic to photosynthetic organisms.

In this study, we identified the target genes of Ssl0564 in *Synechocystis* sp. PCC 6803 by using DNA microarray technique to elucidate the function of small LuxR-type regulators in cyanobacteria.

MATERIALS AND METHODS

Strains and culture conditions. A glucose-tolerant wild-type strain of *Synechocystis* sp. PCC 6803 and the ssl0564-disrupted mutant made by inserting the kanamycin resistance cassette at an *Apa*I site were grown at 32 °C in BG-11 medium with 20 mM HEPES-NaOH, pH 7.0, under continuous illumination at 20 μmol photons $m^{-2} s^{-1}$ provided by fluorescent lamps. Cells were grown in volumes of 50 ml in test tubes (3 cm in diameter) and bubbled by air. Cells at the exponential growth phase ($A_{730} = 0.1$ to 0.2) were harvested for isolation of total RNA.

DNA microarray analysis. Total RNA used for DNA microarray analysis was isolated using the RNeasy Midi kit (Qiagen) as previously described (Hihara et al 2001). After the removal of trace amounts of contaminating genomic DNA by treatment with DNase I (Takara), RNA was labeled with Cy3-dUTP or Cy5-dUTP (Amersham Pharmacia) using RNA fluorescence labeling core kit Ver. 2.0 (M-MLV version; Takara). Hybridization of labeled probes with CyanoCHIP (ver. 1.6; Takara) was performed according to the manufacturer's instruction. Image acquisition with a ScanArray 4000 (GSI Lumonics) and data analysis by a QuantArray version 2.0 software (GSI Lumonics) were performed as previously described (Hihara et al 2003).

RESULTS

Before performing DNA microarray analysis, we checked if ssl0564 is expressed in the wild-type cells under normal growth conditions. We could detect its expression by RT-PCR, although no clear band was observed by Northern blot analysis (not shown). It was suggested that the expression level of ssl0564 was quite low. Then we compared gene expression profile under normal growth conditions between the wild-type and ssl0564-disurupted cells. As shown in Fig. 1, most of genes were expressed similarly in both strains. However, transcript levels of several genes were reproducibly affected by disruption of ssl0564. It was revealed that *ndhD2* encoding a subunit of NAD(P)H dehydrogenase, *rpe* encoding pentose-5-phosphate-3-epimerase and ssl0564-sll0296 operon were up-regulated and *chlL*, *chlN* and *chlB* genes encoding subunits of light-independent protochlorophyllide reductase, *katG* encoding catalase-peroxidase and slr1957 were down-regulated in the mutant under normal growth conditions (Table 1). We searched promoter regions of these putative target genes of Ssl0564 to know whether consensus binding sites for Ssl0564 existed or not. As shown in Fig. 2, multiple inverted repeats of GerE-binding motifs consisting of the 12-mer sequence 5′-RWWTRGGYNNYY-3′ (where R is puRine, W (Weak) is A or T, N is aNy base and Y is pYrymidine) were found at the promoter region of each gene.

DISCUSSION

Putative Target Genes of Ssl0564. It was revealed that Ssl0564 worked as both transcriptional activator and repressor and also regulated its own level. Under normal growth conditions, Ssl0564

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Fey, V., **Wagner, R.**, Bräutigam, K., Wirtz, M., Hell, R., Dietzmann, A., Leister, D., Oelmüller, R., and Pfannschmidt, T. **(2005)**. "Retrograde plastid redox signals in the expression of nuclear genes for chloroplast proteins of *Arabidopsis thaliana*." Journal of Biological Chemistry 280(7): 5318-5328.

Dieser Artikel zeigt auf, dass bei Anpassung an verschiedene Lichtqualitäten die Transkription von plastidären (nachgewiesen mit einem von Vidal Fey angepassten *Primer Extension*-Verfahren) und nukleären Genen (gezeigt mittels Array-Technologie) in *A. thaliana* betroffen sind. Weiterhin wurde gezeigt, dass die Veränderung der Photosystemstöchiometrie bei Lichtqualitätsverschiebung in *A. thaliana* vorwiegend auf einer Mengenveränderung des Photosystem I in Bezug auf die Gesamtproteinkonzentration beruht. Durch Messung des Gehaltes und Redoxzustandes von Glutathion konnte nachgewiesen werden, dass die verwendeten Lichtqualitäten auf dieses Antioxidant-System keinen Einfluss ausüben und damit eine Beteiligung reaktiver Sauerstoffspezies an dieser Reaktion unwahrscheinlich ist.

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Retrograde Plastid Redox Signals in the Expression of Nuclear Genes for Chloroplast Proteins of *Arabidopsis thaliana**[S]

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Excitation imbalances between photosystem I and II generate redox signals in the thylakoid membrane of higher plants which induce acclimatory changes in the structure of the photosynthetic apparatus. They affect the accumulation of reaction center and light-harvesting proteins as well as chlorophylls *a* and *b*. In *Arabidopsis thaliana* the re-adjustment of photosystem stoichiometry is mainly mediated by changes in the number of photosystem I complexes, which are accompanied by corresponding changes in transcripts for plastid reaction center genes. Because chloroplast protein complexes contain also many nuclear encoded components we analyzed the impact of such photosynthetic redox signals on nuclear genes. Light shift experiments combined with application of the electron transport inhibitor 3-(3′,4′-dichlorophenyl)-1,1′-dimethyl urea have been performed to induce defined redox signals in the thylakoid membrane. Using DNA macroarrays we assessed the impact of such redox signals on the expression of nuclear genes for chloroplast proteins. In addition, studies on mutants with lesions in cytosolic photoreceptors or in chloroplast-to-nucleus communication indicate that the defective components in the mutants are not essential for the perception and/or transduction of light-induced redox signals. A stable redox state of glutathione suggest that neither glutathione itself nor reactive oxygen species are involved in the observed regulation events pointing to the thylakoid membrane as the main origin of the regulatory pathways. Our data indicate a distinct role of photosynthetic redox signals in the cellular network regulating plant gene expression. These redox signals appear to act independently and/or above of cytosolic photoreceptor or known chloroplast-to-nucleus communication avenues.

The light environment of plants is highly variable. This is of particular importance for photosynthesis, because changes in incident light intensity or quality can reduce the efficiency of photosynthetic electron transport and therefore the net energy fixation. Plants have developed many acclimatory mechanisms at the molecular level that enable them to cope with such changes. Most prominent responses are dynamic changes in the structure and composition of the photosynthetic apparatus (1–3).

Light quality and quantity gradients that occur *e.g.* in dense plant populations induce an imbalance in excitation energy distribution between the two photosystems (which work electrochemically in series) and therefore reduce photosynthetic efficiency. To counteract such imbalances plants re-distribute light energy in a short term by state transitions (4, 5) and in a long term by a re-adjustment of photosystem stoichiometry. This results in a supply of more light quanta to the less active side of the electron transport chain (6–8). Both processes are regulated by light-induced changes in the redox state of photosynthetic components (9–11). While the short term response acts via post-translational phosphorylation of existing antenna proteins, the long term response (LTR)[1] requires the synthesis of new components and hence has to affect gene expression. This implies signaling routes that connect photosynthetic electron transport/efficiency with the expression machinery. Studies in the last decade show that such functional connections exist at multiple levels and in virtually all classes of photosynthetic organisms. In higher plants photosynthetic redox control has been found at the levels of transcription (12–19), transcript stability (20–23), ribosome loading (24–26), translation initiation (27), and protein accumulation (28).

The origin of the respective signal transduction pathways can be very different. To date three classes of redox signals can be distinguished: the first one is generated directly within the electron transport chain, the second is represented by photosynthesis-coupled redox-active compounds such as thioredoxin or glutathione, and the third is constituted by reactive oxygen species, which are unavoidable by-products of photosynthesis (29–31). Such signals operate within the chloroplast, but have also been shown to affect the expression of some nuclear genes for plastid proteins. Therefore, they may represent a new class of the so-called "plastid signals" (32–35). Retrograde signaling represents an important feedback control that couples the expression of nuclear encoded plastid proteins to the functional state of the chloroplast. Underlying signaling mechanisms in this communication still represent a great field of open questions in plant cell biology. To date neither the impact of retro-

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[S] The on-line version of this article (available at http://www.jbc.org) contains Supplemental Table SI.

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[1] The abbreviations used are: LTR, long term response; Chl, chlorophyll; DCMU, 3-(3′,4′-dichlorophenyl)-1,1′-dimethyl urea; PAM, pulse-amplitude modulation; *psaAB*, chloroplast genes for PsaA and PsaB reaction center proteins of photosystem I; *psbA*, chloroplast gene for reaction center D1 protein of photosystem II; PSI, photosystem I; PSII, photosystem II; PIPES, 1,4-piperazinediethanesulfonic acid; GST, glutathione *S*-transferase; PEP, plastid-encoded RNA polymerase; μE, μmol photons per m^2 and s.

grade redox signals on the nuclear transcriptome of chloroplasts nor possible interaction with other retrograde signals or with photoreceptor-mediated light signals are known while an interaction with sugar signals has been reported (19).

In this study we characterize the role of plastid redox signals in the regulation of plastid and nuclear genes during photosystem stoichiometry adjustment in *Arabidopsis thaliana*. By the use of this model organism we take advantage from the mutant and array resources available for this organism offering experimental strategies, which are not possible with tobacco and mustard used in earlier studies (9, 14). We describe for the first time the molecular response to PSI or PSII light in chloroplasts of *A. thaliana.* Determinations of glutathione content and redox state were performed to check possible interactions of different redox signals in this event. Cross-talk of the LTR with other signaling routes has been tested in mutants lacking either photoreceptors or components of plastid-to-nucleus signal pathways. By using a macroarray approach we determined the impact of plastid redox signals on the nuclear transcriptome of chloroplasts. Our study indicates that chloroplast redox signals from the thylakoid membrane represent a novel and separate class of plastid signals.

EXPERIMENTAL PROCEDURES

Plant Growth—Plants were grown in temperature-controlled growth chambers at 22 °C under continuous light. *Arabidopsis* seeds (var. Col 0 or *Landsberg erecta* and mutant lines in the respective backgrounds) were sown either sterile on half-strength Murashige and Skoog (MS) medium containing 1.35% sucrose or on earth substituted with vermiculite. Density of seeds was adjusted in such a way that 16-day-old plants did not shadow each other. After 2 days at 4 °C plants were grown for 10 days under white light provided by 30-watt white stripe lamps (OSRAM, München, Germany) with a photosynthetic-active radiation of ~35 μE. This white light pre-treatment was found to be necessary for the plants to develop a normal leaf anatomy and hence a true acclimatory response. Direct germination and growth under the PSI or PSII light sources resulted in aberrant leaf anatomy due to the lack of blue radiation of these light sources. After growth in white light, plants were acclimated to PSI (photosynthetic active radiation, ~20 μE) or PSII (photosynthetic active radiation, ~30 μE) light for 6 days or they were first acclimated to one light source for 2 days followed by 4 days under the respective other light source. PSI and PSII light sources have been described earlier (9, 12); however, the incandescent bulbs of the PSI light source were replaced by 18-watt fluorescent stripe lamps "Red" (OSRAM, München, Germany) of the same photon flux density to reduce thermal radiation. The photosynthetic active radiation was determined by using the lightmeter LI-250 (Heinz Walz GmbH, Effeltrich, Germany). It must be noted that the far-red spectrum of the PSI light is outside of the detection range of the LI-250. White light control plants were grown for 16 days under the white light source alone.

Chlorophyll Fluorescence Measurements—In vivo Chl *a* fluorescence parameters were determined at room temperature with a pulse amplitude-modulated (PAM) fluorometer (PAM101/103, Heinz Walz). 10–15 seedlings grown on MS medium were measured simultaneously as described previously (14). After dark acclimation (8–10 min) the measuring beam was turned on, and minimal fluorescence (F_o) was determined. Then leaves were exposed to a 500-ms flash of saturating white light (6000 μE) to determine maximal fluorescence (F_m) and the optimum quantum yield F_v/F_m value was calculated as $F_m - F_o/F_m$ (36). Subsequently, leaves were illuminated with 100 μmol of photons m^{-2} s^{-1} of actinic red light of 600 nm (Walz 102-R). Fluorescence was recorded in the saturation pulse mode by application of saturating flashes every 30 s to determine maximal fluorescence of illuminated leaves (F_m') until a stable fluorescence level (F_t) was reached. Actinic light was switched off, and far-red light (Walz 102-FR) was turned on to oxidize the electron transport chain and to determine minimal fluorescence (F_o') in the light-acclimated state. The steady-state fluorescence F_s was then calculated as $F_t - F_o' = F_s$. The optimum quantum yield describes the maximal photosynthetic capacity of a plant and was taken as a measure for photosynthetic efficiency of the mutant lines analyzed in this study in comparison to wild type. For wild type we found F_v/F_m values of 0.8–0.83, which typically indicate that the plant analyzed has no decreased photosynthetic efficiency. Only plants with a wild type like behavior were tested for their response to the two light sources. A proper acclimation response to PSI or PSII light is characterized by a significant change in the F_s/F_m value as shown earlier (14) and reflects the structural differences in the photosynthetic apparatus of these plants. The difference of 10 μE in photosynthetic active radiation between PSI and PSII light has no detectable impact on this acclimation, because in control experiments PSI plants showed the same decrease in F_s/F_m after acclimation to either 20 or 30 μE PSII light (data not shown). One-way analysis of variance was used to reveal significant differences in F_s/F_m values of plants grown under the defined conditions. Light treatment was used as a factor, and the F_s/F_m value as a dependent variable. If a significant influence of light treatment was determined, post-hoc tests (pairwise multiple comparison test for lowest significance difference) was performed to find out which groups differ from each other. $p < 0.5$ determines significant differences between various samples (see Supplementary Table SI). All tests were performed using SPSS 11.5.

Chlorophyll Content Determination—Total chlorophyll was determined spectroscopically after grinding of leaves in liquid nitrogen and extracting chlorophylls with 80% (v/v) buffered acetone. Concentrations of chlorophylls *a* and *b* were calculated by using the extinction coefficients from previous studies (37).

Western Analyses of Chloroplast Proteins—20 g of leaf material of plants grown on soil were harvested under the respective light source and directly homogenized in ice-cold buffer containing 0.05 M Hepes/KOH, pH 8.0, 0.33 M sorbitol, 0.001 M $MgCl_2$, and 0.002 M EDTA. The material was filtered through four layers of muslin and one layer of Miracloth, followed by a centrifugation (10 s at 6000 rpm). The pellet was washed twice in homogenization buffer and resuspended in 1 ml of the same buffer. Concentrations of chloroplasts were determined microscopically by counting diluted aliquots in a Fuchs-Rosenthal chamber. 2×10^5 plastids of each preparation were lysed and denatured in 5× SDS sample buffer (final concentrations: 0.4% SDS, 0.1% β-mercaptoethanol, 2% glycerol, 0.02% bromphenol blue) by incubation for 5 min at 95 °C. Insoluble particles were removed by centrifugation, and samples were loaded on denaturing 10% SDS-polyacrylamide gels (38) and separated overnight at 45 V. Proteins were transferred to a nylon membrane (Roti-Nylon Plus, Roth, Karlsruhe, Germany) at 400 mA for 1 h using a semi-dry blotting apparatus, and the membrane was saturated in Tris-buffered saline containing 2% fat-free milk powder. Incubation with polyclonal antisera followed standard protocols (39). Antibodies for D1, Lhca3, and Lhcb1 were purchased from AgriSera (Vannas, Sweden). Detection of the first antibody was performed with a goat-anti-rabbit-IgG-peroxidase conjugate (Sigma, München, Germany) and the enhanced chemiluminescent (ECL) detection system. For visualization of marker proteins and to prove blotting efficiency, membranes were stained with Amido Black (39) after the ECL reaction.

RNA Preparations—RNA for primer extension analyses was isolated from plants grown on MS medium. RNA for array analyses was isolated from wild-type (Col-0) plants grown on soil. Leaf material was harvested and frozen in liquid N_2 under the respective light source. Total RNA was isolated using the TRIzol reagent (Invitrogen) following a protocol described earlier (40). Concentration and purity of RNA samples were determined spectroscopically in a Biophotometer (Eppendorf, Hamburg, Germany). Intactness was proven by ethidium bromide staining of rRNA species after electrophoretic separation of aliquots on denaturing 1.2% agarose gels containing formaldehyde (39). Isolated RNA was stored at −80 °C until further use.

Primer Extension Analyses—Primer extension analyses (41) were carried out according to a protocol from Li-Cor (Bad Homburg, Germany). 5 μg of total RNA was resolved in 20 μl of hybridization mixture containing 1.25 μM infrared dye 700-labeled *psaA*-specific and infrared dye 800-labeled *psbA*-specific primers and 18 μl hybridization buffer (50% formamide, 1 mM EDTA, 400 mM NaCl, 40 mM PIPES, pH 6.4). After denaturation at 80 °C for 15 min RNA/primer hybrids were allowed to form at room temperature for 1 h. Hybrids were precipitated with 2.5 volumes of 96% EtOH at −80 °C for 30 min and washed with 100 μl of 70% EtOH. Precipitates were dried and resolved in 2 μl of 5× buffer for Moloney murine leukemia virus reverse transcriptase, 4 μl of 5 mM dNTPs, 3 μl of H_2O, and 1 μl of Moloney murine leukemia virus reverse transcriptase (MBI Fermentas, 200 units/μl)), and incubated 1 h at 42 °C. 1 μl of the samples was mixed 1:1 with formamide loading dye (Amersham Biosciences), applied onto a sequencing gel (4% acrylamide, 1× TBE (0.089 M Tris, 0.089 M boric acid, 0.002 M EDTA, ph 8.0), 7 M urea, 66 cm × 0.25 mm) and separated according to Li-Cor (Bad Homburg, Germany) recommendations. Gene-specific primer sequences: *psaA*, 5′ infrared dye 700, 5′-CCC ATT CCT CGA AAG-3′ (sequence position +65 to +79 relative to ATG); *psbA*, 5′ infrared dye 800, 5′-AGA CGG TTT TCA GTG-3′ (sequence position +69 to +83

relative to ATG). The same primers were used to sequence the respective region of *Arabidopsis* chloroplast DNA using a cycle sequencing kit (MBI Fermentas, St. Leon-Roth, Germany).

Determination of Thiol Group Content and Redox State of Glutathione—For isolation of total glutathione and cysteine 25 mg of leaf material was ground in liquid N_2 and extracted with 0.5 ml of buffer E (100 mM phosphate, pH 7.1, 50% methanol, 5 mM dithiothreitol) for 10 min at 60 °C while shaking. Homogenates were centrifuged twice at 15,400 × *g* for 5 min at room temperature, and supernatants were used for further analysis. Determination of oxidized glutathione was based on the same extraction, but dithiothreitol in buffer E was replaced by 5 mM *N*-ethylmaleimide to block reduced glutathione (42). Reduction of oxidized thiols in the extracts (0.02 ml) was carried out at room temperature for 60 min in a total volume of 0.27 ml containing 134 mM Tris, pH 8.3, 1 mM dithiothreitol. Then thiols were derivatized for 15 min by adding 0.03 ml of monobromobimane (Calbiochem, La Jolla) to a final concentration of 3 mM (2.5-fold excess above total thiol concentration). Resulting monobromobimane derivatives were stabilized by addition of 0.7 ml of 5% acetic acid and detected fluorometrically (Fluorometer RF 551, Shimadzu) at 480 nm by excitation at 380 nm after separation by reverse-phase HPLC using a Waters HPLC-system (Waters 600E Multisolvent Delivery system, Autosampler 717plus) connected to a Nova-Pak C18 4.6 × 250-mm column (pore size, 4 μm). Glutathione and other thiols were separated by applying an isocratic flow (1.3 ml/min) of buffer A (100 mM potassium acetate, pH 5.5, 9% methanol) for 12.5 min. The column matrix was washed with 100% methanol for 3 min and re-equilibrated for 8.5 min in buffer A. Data acquisition and processing was performed with Millenium^{32} software (Waters). Reduced glutathione concentrations were calculated from the difference between total and oxidized glutathione. Recovery rates were higher than 95% for reduced and oxidized glutathione and higher than 90% for cysteine, respectively, as determined by spiking of samples with internal standards. Samples were analyzed in quadruplicate.

Expression Profiling—The 3292-GST nylon array, including 2661 nuclear chloroplast genes and 631 genes coding for non-chloroplast proteins, has been described previously (43). Experiments were performed with plant material corresponding to pools of at least 250–500 individuals. To obtain larger amounts of tissue of healthy and unstressed plants, seedlings were initially grown 22 days under white light (short day periods, 8-h light/16-h dark) on soil. Plants were then acclimated to: (i) PSI light (5 days), (ii) PSI light (3 days) followed by PSII light (2 days), (iii) PSI light (3 days) followed by PSII light plus 5 μM DCMU (2 days), or (iv) PSI light plus 5 μM DCMU. DCMU (Sigma) has been applied to plants directly before performing the light shifts using a fine sprayer as described before (14). DCMU stock solution was 10 mM in 50% ethanol, and the applied concentration was prepared by dilution in sterile water directly prior use. The drug was found to be completely stable during the 2-day period of experiment as determined by the effect on chlorophyll *a* fluorescence using a PAM101 fluorometer. Effects of DCMU on photosynthetic electron flow have been proven by determination of ΦPSII (44) at the end of the treatments (PSI: 0.72 ± 0.02; PSI–II: 0.8 ± 0.02; PSI–II plus DCMU: 0.49 ± 0.05; PSI plus DCMU: 0.53 ± 0.05). Three independent experiments with different filters and independent cDNA probes were performed thus minimizing variation between individual plants, filters, or probes. cDNA synthesis was primed by using a mixture of oligonucleotides matching the 3292 genes in antisense orientation and hybridized to the GST array as described (43, 45). Images were read using a Storm PhosphorImager (Amersham Biosciences). Hybridization images were imported into the ArrayVision program (version 6, Imaging Research Inc., Ontario, Canada), where artifacts were removed, background correction was performed, and resulting values were normalized with reference to intensity of all spots on the array (45). In the next step, those data were imported into the ArrayStat program (version 1.0 Rev. 2.0, Imaging Research Inc.), and a *z*-test (nominal α set to 0.05) was performed employing false discovery rate (46) correction to identify statistically significant differential expression values. Only differential expression values fulfilling the criteria of this statistical procedure were used for the expression profiling.

RESULTS

Changes in Photosystem Structure of Arabidopsis during Acclimation to PSI and PSII light and Transcriptional Regulation of Plastid Reaction Center Genes psaA and psbA—Imbalances in excitation energy distribution between the photosystems can be induced by illumination with light sources that differentially excite PSII or PSI (PSII or PSI light, respectively) resulting either in a more reduced or more oxidized state of the electron transport components (data not shown). To study how plants deal with and acclimate to such imbalances, *Arabidopsis* seedlings were grown first under white light until the four- to six-leaf stage before they were subjected to PSI or PSII light (PSI or PSII plants). Responses of such plants were compared with responses of plants acclimated to PSI or PSII light followed by an additional acclimation to the respective other light source (PSI–II plants or PSII–I plants). The analysis of PSI and PSII plants show the acclimation to the two light sources in general, whereas the analysis of the plants shifted between the light sources proves the reversibility of the observed responses (an indicator for true acclimatory effects). To test photosystem stoichiometry adjustment in response to light quality in *Arabidopsis* we analyzed photosystem protein abundance and chlorophyll contents. The overall protein pattern of whole tissue protein extracts did not reveal any major differences between the four growth conditions when analyzed by SDS gel electrophoresis. In Western analyses with antisera raised against the D1 protein and the P700 apoproteins (representing the core proteins of PSII and PSI) (Fig. 1*A*) the D1 protein exhibited more or less constant amounts under all conditions, whereas the amounts of P700 apoproteins increased in PSI–II plants in

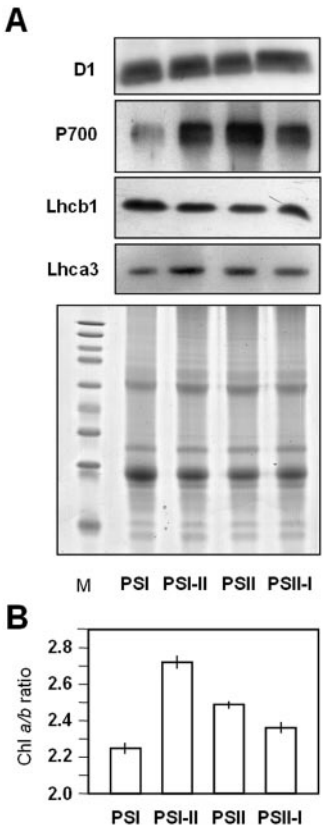

FIG. 1. **Changes in photosynthesis protein and chlorophyll amounts during long term acclimation.** *A*, Western immunological analysis of photosystem core and antenna protein content. Chloroplasts of the differentially grown plants were isolated, and proteins of ~2 × 10^5 organelles were separated by SDS-PAGE per lane and transferred to nylon membranes. Respective growth conditions are given at the *bottom*. D1 protein, P700 apoproteins, Lhcb1, and Lhca3 were detected with polyclonal antisera and a peroxidase-coupled secondary antibody using enhanced chemiluminescence. Representative results from three independent experiments are shown. A Coomassie Blue-stained SDS gel is shown below as loading control. Marker proteins (*lane M*) range from 116 to 14 kDa. Growth conditions are given at the *bottom*. *B*, Chl *a/b* ratio. Chlorophylls of acclimated plants were extracted, spectrophotometrically determined, and calculated as described under "Experimental Procedures." Growth conditions are given at the *bottom*. All experiments were repeated three times.

comparison to PSI plants and decreased in PSII–I plants in comparison to PSII plants. Taking the amount of the reaction center proteins as an indicator for the relative number of the photosystems, the PSII/PSI ratio is high under PSI light and decreases after a shift to PSII light, whereas the opposite can be observed under PSII light and a shift to PSI light. Furthermore, we tested the abundance of antenna proteins Lhcb1 and Lhca3, two important components of the PSII and PSI antennae, respectively. Lhca3 showed a similar accumulation under the light sources as the P700 apoproteins, whereas the opposite effect was observed for the Lhcb1 protein suggesting a concomitant increase in antenna size of the respective rate-limiting photosystem. Such changes in the antennae are also indicated by characteristic changes in the Chl *a/b* ratio. After acclimation to PSI light the Chl *a/b* ratio is low and increases significantly after a shift to PSII light (Fig. 1*B*, PSI–II). Under PSII light the Chl *a/b* ratio is high and decreases after a shift to PSI light. Because Chl *b* is mainly associated with the PSII antenna, these observations are consistent with the observed changes in the amounts of antenna proteins.

In mustard the adjustment of photosystem stoichiometry is controlled by changes in the transcription of the reaction center genes *psaA* and *psbA* (9, 12). To test if this is also true for *Arabidopsis* we performed primer extension analyses (Fig. 2) for these genes that allowed us to check for changes in transcript initiation sites and amounts of the respective RNAs in the same experiment. Both *psaA* and *psbA* transcripts exhibited the same 5′-ends under all conditions investigated, although in varying amounts. For *psaA* we found two prominent 5′-ends in a distance of 197 and 111 bases upstream of the translation initiation codon. The first (more prominent) end corresponds to the transcription start sites for *psaA* in mustard (47), the second one has not been reported in any other organism and might represent a species-specific start or processing site. The regulation of *psaA* transcript accumulation is comparable to the situation observed for the respective proteins with an increase in transcripts after a PSI–II light shift (in comparison to PSI light) and a decrease after a PSII–I light shift (in comparison to PSII light). For *psbA* we found a single prominent 5′-end 78 bases in front of the translation start site, consistent with earlier reports (27, 47, 48). The accumulation of this transcript showed only a slight decrease after a PSI–II light shift and a slight increase after a PSII–I light shift. The observed changes in RNA amounts are in agreement with the observations at protein level suggesting that redox-regulated transcription plays an important role also in *Arabidopsis*.

Light Quality Acclimation in Photoreceptor and Chloroplast-to-Nucleus Signaling Mutants—Adjustment of photosystem stoichiometry in higher plants requires coordinated changes in the expression of plastid- and nuclear-encoded photosynthesis genes. To test whether cytosolic photoreceptors or components of plastid retrograde signaling pathways are involved in the detection and/or transduction of PSI or PSII light-induced redox signals, we analyzed the LTR in various *Arabidopsis* mutants (Fig. 3). We used the Chl fluorescence parameter F_s/F_m, which in wild type typically increases after acclimation to PSI light and decreases after acclimation to PSII light (13, 14)[2] and, therefore, can be used as a non-invasive indicator for a LTR. In the photosynthesis mutant *hcf109* (49), which exhibits partial impairment of PSII and PSI activities, no significant changes in the F_s/F_m values could be observed (data not shown) indicating that perturbations of photosynthetic electron transport lead to a loss of the LTR and/or its detectability. Therefore, before assessing the F_s/F_m value each mutant line was tested

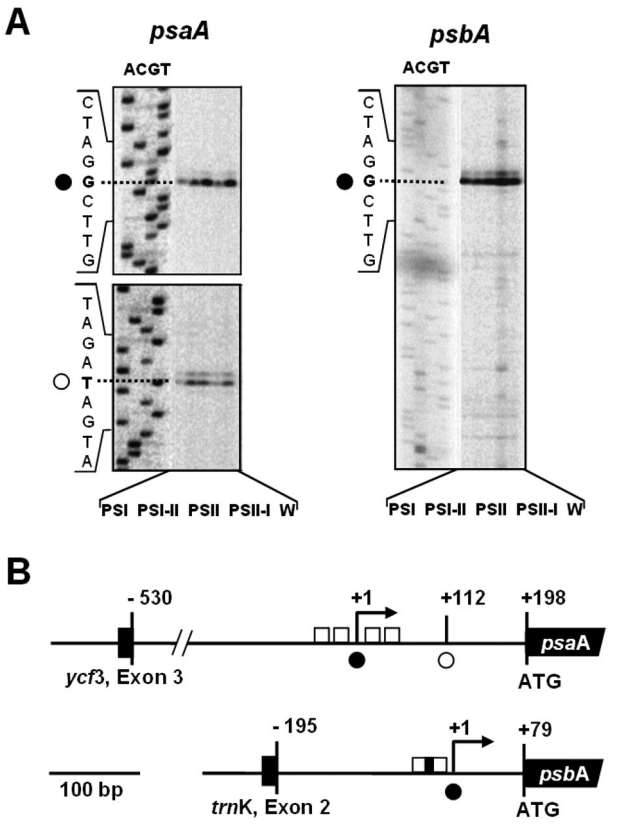

FIG. 2. **Primer extension analysis of position and accumulation of 5′- ends of *psaA* and *psbA* transcripts.** Plants were grown under the respective light sources, and total RNA was isolated. Fluorescence dye-labeled primers were designed to anneal within the first 50 bp of the coding region of the *psaA* and *psbA* genes and were used both in a reverse transcription reaction with isolated total RNA and a sequencing reaction of chloroplast DNA fragments covering the *psaA* and *psbA* 5′-gene and promoter regions. Products were separated in parallel on a denaturing 4% acrylamide gel containing 7 M urea and detected by laser excitation in a Licor 4200 sequencer. *A*, sequencer images of the primer extension analyses. The DNA sequences within the *psaA* (*left part*) and *psbA* (*right part*) promoters are shown each on the *left*, primer extension products on the *right*. Detected 5′-ends are marked by *dots* (*black* for transcript start; *white* for unknown end), and respective transcription start nucleotides are given in *bold letters*. Growth conditions are given at the *bottom*. *B*, structure of the *Arabidopsis psaA* and *psbA* promoter regions. Positions of 5′-ends are marked by the same *dots* as in Fig. 5*A*. Transcription start sites are indicated by +1, and all other positions are given relative to it. Pairs of *white boxes* indicate −10/−35 regions; a *black box* indicates a TATA-like *cis*-element.

for its Chl fluorescence parameter F_v/F_m as indicator for the general photosynthetic function. All mutants revealed wild type-like F_v/F_m values of >0.8 (data not shown) indicating that they can perform normal photosynthesis. We then tested the LTR in mutants lacking functional phytochrome A (*phyA*), phytochrome B (*phyB*), or both (*phyA*/*phyB*) (50) as well as for a transgenic line overexpressing phytochrome B (*phyB oe*) (51). In addition, we tested mutants lacking cryptochrome 1 (*hy4*) or 2 (*cry2-1*) (52, 53). A significant decrease or increase of F_s/F_m after the respective light switch was observed for all photoreceptor mutants indicating their ability to perform an appropriate LTR (Fig. 3*A*). Only the *phyA*/*phyB* double mutant revealed no significant decrease of the F_s/F_m value after a shift from PSI to PSII light, whereas the *cry2–1* mutant exhibited a significant LTR, however, with a less strong increase in F_s/F_m than usually observed after a shift from PSII to PSI light (compare Supplementary Table SI).

We also analyzed the response of *genome-uncoupled* (*gun*) (54) and *cab underexpressed* (*cue*) (55) mutants (Fig. 3*B*). Both types of mutants exhibit defects in chloroplast signaling routes

[2] R. Wagner and T. Pfannschmidt, unpublished observations.

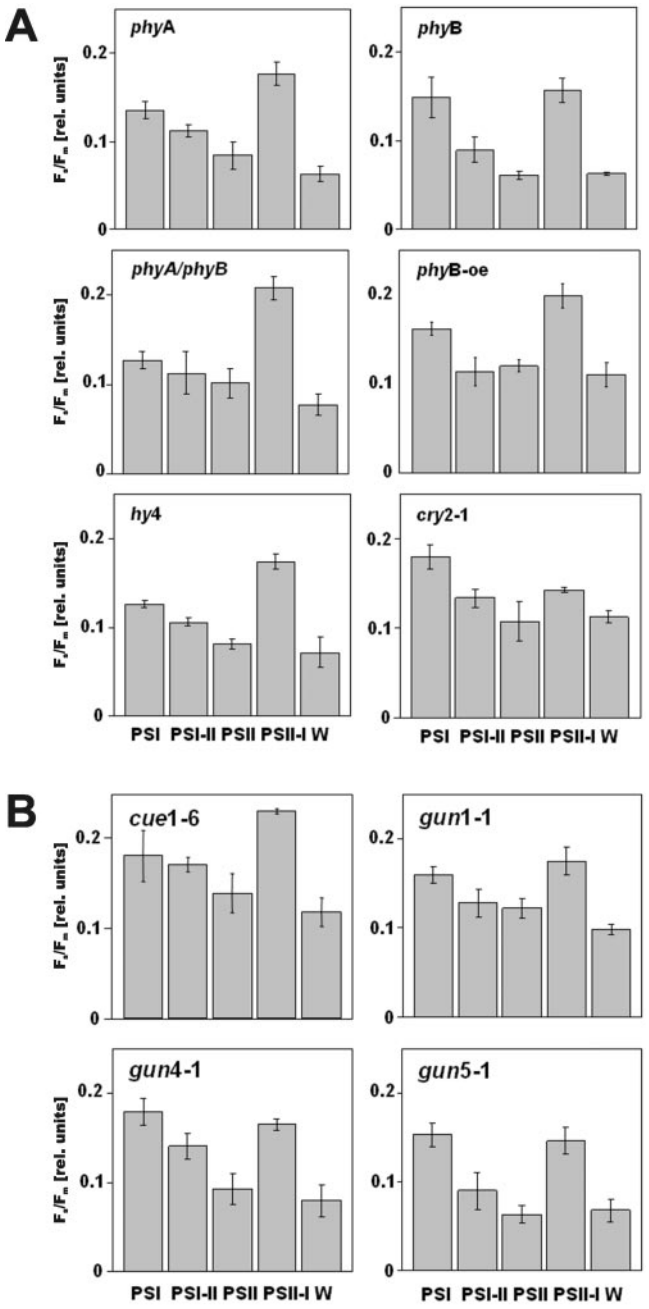

FIG. 3. **LTR in *Arabidopsis* mutants.** Mutant lines were acclimated to PSI or PSII light, and F_s/F_m values were determined using a PAM fluorometer. All values were determined in at least three independent experiments with 15–20 plants each, and the statistical significance of differences was proven using the SPSS statistic program (for details see Supplementary Table SI). The indication of the respective lines is given in the *upper left corner* of each graph (for designation see text). *A*, photoreceptor mutants; *B*, chloroplast-to-nucleus signaling mutants.

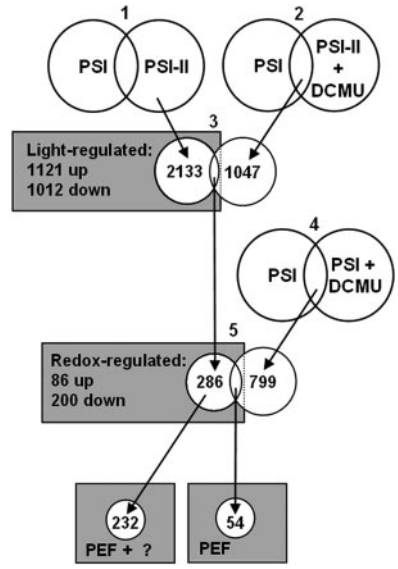

FIG. 4. **Macroarray strategy to define redox-regulated genes encoding chloroplast proteins.** White light-grown plants were acclimated to PSI light for 6 days (*PSI*) or 2 days followed by an acclimation to PSII light for additional 4 days (*PSI–II*). Parallel samples were treated with 5 μM DCMU after 2 days in PSI light and then shifted to PSII light or left under the PSI light. *Large circles* represent the respective expression profiles (test condition is given inside). Profiles of these conditions (*large circles*) were compared (for details see text). Intersections represent genes that do not differ significantly in their expression under the conditions compared. *Small circles* represent gene groups resulting from these comparisons (number is given inside), and their respective origin is indicated by *arrows*. *Gray boxes* list category and number of up- or down-regulated genes in the *small circles* that originate from comparisons between expression profiles represented by *large circles* (for details, see text). PEF, genes that are regulated by photosynthetic electron transport. PEF + ?, genes that are regulated by photosynthetic electron transport and an unknown additional redox signal from the thylakoid membrane.

toward the nucleus. *gun1-1* has still unknown defects, *gun5* encodes the H subunit of the magnesium chelatase in the chloroplast envelope, and *gun4* encodes a product that binds the substrate of the magnesium chelatase (56, 57). The *cue1* gene encodes the phosphoenol pyruvate/phosphate translocator of the chloroplast envelope (58). In our test system all *gun* mutants exhibited a wild type-like behavior with significant LTRs. The *cue1-6* mutant, however, showed no significant decrease of F_s/F_m after a shift from PSI to PSII light, whereas the expected increase after a shift from PSII to PSI light is present to a full extent (compare Supplementary Table I).

These data indicate that the defective components in the photoreceptor and retrograde signaling mutants are not essential for the LTR, otherwise we would have observed a complete loss of it. Thus, chloroplast redox signals represent a unique class of retrograde signals. The less pronounced effects in *phyA*/*phyB* and *cue1-6* mutants might be caused by general developmental effects (see "Discussion") suggesting that redox signals are an integral component of the intracellular signaling network.

Photosynthetic Control of the Nuclear Transcriptome of the Chloroplast—To analyze the global effects of light quality and redox signals on the expression of genes for chloroplast proteins, we performed a macroarray analysis using a GST array with probes covering respective nuclear genes (45). This preselection of genes guarantees that a high proportion of light-regulated genes are investigated. Light regulation is a prerequisite for the study of redox regulation under our conditions. Furthermore, this array has been shown in earlier studies to produce statistically reliable and reproducible expression profiles (43, 59). To assess the impact of redox signals we followed a three-step strategy. 1) First we compared gene expression profiles of PSI and PSI–II plants (Fig. 4, *comparison 1*). This showed the overall impact of a reduction signal induced by the shift from PSI to PSII light. Non-light-regulated genes could be identified and omitted from further analysis. 2) Next we compared gene expression profiles of PSI plants with PSI–II plants pre-treated with DCMU (Fig. 4, *comparison 2*). Genes with the same expression under both conditions represent either non-light-regulated genes or light-regulated genes whose expression change is abolished by the electron transport inhibitor.

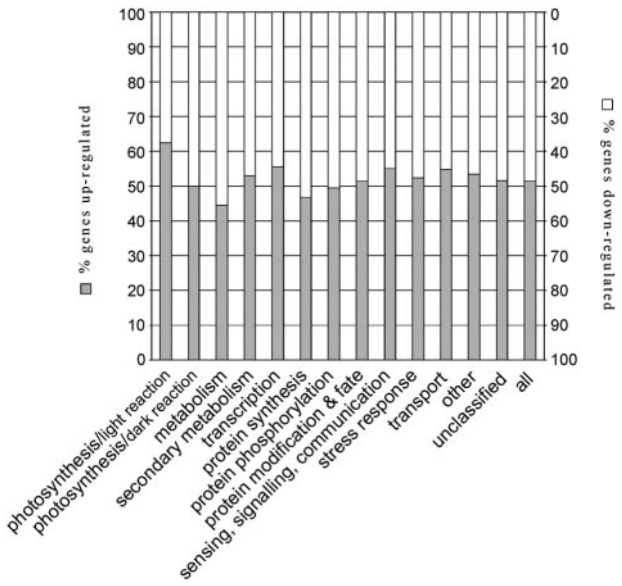

FIG. 5. **Relative distribution of light quality-induced expression changes of genes sorted by function of gene product.** Only genes with significant expression changes were included. Genes were grouped according to the known or predicted function of the encoded product (given at the *bottom*). Numbers of genes with increased (*gray part of bar*) or decreased (*white part of bar*) expression are given in percentages.

The latter are defined as redox-regulated genes and could be identified by comparing this group of non-regulated genes with those responsive to the light signal from step 1 (Fig. 4, *comparison 3*). 3) Finally, we compared gene expression profiles of PSI plants and PSI plants treated with the same amounts of DCMU as in step 2 (Fig. 4, *comparison 4*). Redox-regulated genes whose expression change is completely abolished by the DCMU treatment were controlled by the photosynthetic electron flow when the same DCMU treatment as in step 2 had no effect in step 3 indicating that the DCMU treatment has only neutralized the PSII light effect. Such "ideal" redox-regulated genes are defined by a comparison of non-regulated genes from comparison 4 with the group of redox-regulated genes of comparison 3 (Fig. 4, *comparison 5*).

Comparison 1 indicates that a light quality shift has a massive impact on the expression of genes encoding chloroplast proteins. A set of 2133 genes significantly responded to the shift from PSI to PSII light; 1121 genes were up-regulated while 1012 were down-regulated. Among these we found genes for all major functional classes of proteins (Fig. 5), including genes for photosynthesis, gene expression, metabolism, and transport. We found no gene class exhibiting unidirectional expression changes. As a general tendency it emerged that all gene classes responded in a balanced way with around 50% up- and 50% down-regulated genes. Out of the 2133 light-regulated genes, we identified 286 that are directly regulated by redox signals from the photosynthetic electron transport chain. 86 genes were up-regulated by a reduction signal while 200 genes were down-regulated by it. From these 286 redox-regulated genes 54 matched the theoretical constraints for an "ideal" expression profile to be expected for a gene regulated by redox signals from photosynthetic electron flow. The remaining 232 genes still represented redox-regulated genes but seemed to be regulated by more than one redox parameter (see "Discussion").

Only 76 of the 286 redox-regulated genes encode products with known functions, including all major gene groups such as photosynthesis, gene expression, metabolism, or signal transduction (Table I). The great majority of genes, however, codes for putative, hypothetical, or even unknown proteins (not shown). Nevertheless, several groups of functionally related genes can be identified that exhibit similar expression patterns pointing to concerted regulatory events. The largest groups among the down-regulated genes include: (*a*) a large group of metabolic genes mainly encoding enzymes (or enzyme subunits) involved in amino acid or nucleotide metabolism; (*b*) several chaperones and signal recognition particle components partially involved in photosystem assembly; (*c*) genes for transcription and its regulation in the nucleus and chloroplast; and (*d*) genes for components involved in sulfur and glutathione metabolism. The largest groups among the up-regulated genes include (*a*) metabolic genes for amino acid and nucleotide metabolism as well as energy metabolism and (*b*) photosynthesis genes. Beside these major groups many individual genes encoding products with functions necessary for the establishment of the LTR are identified (see "Discussion"). This result demonstrates that redox signals from the thylakoid membrane have an extensive influence on the expression of nuclear genes reflecting the multiple functional involvements of chloroplasts within the metabolic pathways of the cell.

Effects of PSI and PSII Light on Thiol Group Content and Glutathione Redox State—Glutathione is an important cellular redox buffer and functions also as a potent regulator of gene expression especially in chloroplasts (16, 17, 22, 60). The array analysis exhibited several regulated genes involved in glutathione metabolism. To test if changes in glutathione redox state are involved in the LTR, we determined the content of cysteine and glutathione as well as the redox state of glutathione in plants (Table II) grown under the four different conditions. We found comparable thiol contents under all growth conditions, and only PSII plants exhibited slightly increased amounts in glutathione and cysteine. In addition, glutathione appeared to be mainly reduced (around 90%) under each light regime, as it is described for *Arabidopsis* grown under standard white light sources. Therefore it is unlikely that changes in the glutathione redox state are responsible for the observed changes in plastid gene expression. Aside from this, the highly reduced state indicates that the plants do not suffer from strong reactive oxygen species-mediated stresses, which is typically indicated by an increase in oxidized glutathione concentrations. Thus, superimpositions from reactive oxygen species-induced redox signaling cascades under the different light qualities are unlikely, and we conclude that in *Arabidopsis* light quality changes are reported mainly via redox signals from intersystem electron transport components.

DISCUSSION

Light Quality Effects on Photosystem Stoichiometry—We found significant acclimatory changes in the structure of the photosynthetic apparatus in an extent comparable to those reported for other higher plants (9, 14, 61, 62). Our Western analyses, however, suggest that photosystem stoichiometry adjustment in *Arabidopsis* is mainly regulated by changes in PSI complexes and PSII antenna size. This differs from observations in pea and mustard were antiparallel changes in both PSI and PSII were observed (12, 61), whereas it is in accordance with observations in spinach and cyanobacteria for which mainly changes in PSI were reported (7, 62). Changes in PSII content in *Arabidopsis* have been reported to occur only under higher light intensities (63). Spectroscopic analyses might help to determine more precisely the absolute changes in photosystems in *Arabidopsis* under our conditions. The immunologically detected changes in D1 and P700 apoprotein levels are accompanied by corresponding changes in respective transcript pool sizes as observed earlier (9, 12). At both promoters redox regulation occurs at the major transcription start site (Fig. 2), which is located directly behind typical promoter elements for

TABLE I
Redox-regulated genes encoding known products

ATG[a]	Ratio[b] PSII/PSI	Ratio[b] PSII_DCMU/PSI	Ratio[b] PSI_DCMU/PSI	Description[c]
Down-regulated				
Metabolism				
At5g38530	0.63	0.81	0.49	Tryptophan synthase, β chain
At4g16700	0.64	0.85	0.72	Decarboxylase-like protein
At2g43090	0.69	0.82	0.71	3-Isopropylmalate dehydratase, small subunit
At5g13280	0.69	0.88	0.45	Aspartate kinase
At4g27070	0.70	0.96	0.54	Tryptophan synthase, β-subunit (TSB2)
At4g16800	0.71	0.85	0.50	Enoyl-CoA hydratase
At4g19710	0.72	0.81	0.51	Aspartate kinase-homoserine dehydrogenase-like protein
At5g03650	0.72	0.88	0.48	1,4-α-glucan branching enzyme isoform SBE2.2
At3g10050	0.73	0.81	0.57	Threonine dehydratase/deaminase (OMR1)
At4g18440	0.74	0.82	0.57	Adenylosuccinate lyase-like protein
At4g09740	0.74	1.03	0.51	Cellulase-like protein
At5g08300	0.76	0.81	0.65	Succinyl-CoA-ligase, α subunit
At4g11010	0.76	0.82	0.47	Nucleoside diphosphate kinase 3 (ndpk3)
At2g03220	0.79	0.85	0.50	Xyloglucan fucosyltransferase AtFT1
At4g31180	0.76	0.89	0.40	Aspartate-tRNA ligase-like protein
Other				
At4g35770	0.68	1.15	2.01	Senescence-associated protein sen1
At5g18810	**0.77**	**1.05**	**0.99**	**Serine/arginine-rich protein-like**
At5g25380	0.77	0.92	0.62	Cyclin 3a
At5g24020	0.80	0.95	0.76	Septum site-determining MinD
Photosynthesis				
At4g15530	0.69	0.80	0.54	Pyruvate, orthophosphate dikinase
At1g76450	0.78	0.86	0.27	Unknown thylakoid lumen protein, PsbP domain
Protein modification and fate				
At2g39990	0.59	0.82	0.50	26 S proteasome regulatory subunit
At5g15450	0.73	0.81	0.70	ClpB heat shock protein-like
At4g36040	**0.75**	**0.90**	**1.25**	**DnaJ-like protein**
At2g28800	**0.76**	**0.86**	**0.98**	**Chloroplast membrane protein ALBINO3 (ALB3)**
At5g03940	0.79	0.96	0.51	Signal recognition particle 54CP (SRP54) protein
At4g37910	0.79	0.85	0.51	Hsp70.3
Protein phosphorylation				
At4g23650	**0.64**	**0.83**	**1.19**	**Calcium-dependent protein kinase (CDPK6)**
Stress response				
At4g29890	**0.62**	**1.16**	**0.89**	**Choline monooxygenase-like protein**
At3g45140	**0.76**	**0.82**	**0.81**	**Lipoxygenase AtLOX2**
Transcription				
At1g59940	**0.75**	**0.87**	**0.90**	**Response regulator ARR 12**
At3g57040	0.79	0.96	0.54	Response regulator ARR 9
RpoB	**0.62**	**1.05**	**1.05**	**Plastid gene; RNA polymerase catalytic chain**
At5g24120	0.63	0.81	0.67	Sigma-like factor (emb CAA77213.1)
At3g56710	0.73	0.88	0.38	SigA-binding protein
At3g60490	0.73	0.92	0.39	Transcription factor-like protein
At1g68990	0.75	0.99	0.51	DNA-directed RNA polymerase (mitochondrial)
At1g03970	0.79	0.81	0.69	G-box binding factor, GBF4
Transport				
At4g33650	0.68	1.03	0.35	*Arabidopsis* dynamin-like protein ADL2
At4g18290	0.68	0.94	0.51	Potassium channel protein KAT2
At4g36580	**0.75**	**1.04**	**0.92**	**ATPase-like protein**
At5g59030	0.76	0.90	0.53	Copper transport protein
Unclassified				
At2g13870	0.79	1.06	0.65	En/Spm-like transposon protein
S-metabolism				
At4g02520	**0.57**	**0.85**	**0.91**	**Atpm24.1 glutathione *S*-transferase**
At5g56760	0.66	0.81	0.47	Serine *O*-acetyltransferase (EC 2.3.1.30) Sat-52
At4g39940	**0.67**	**1.23**	**0.95**	**Adenosine-5-phosphosulfate kinase**
At5g43780	0.75	0.97	0.72	ATP sulfurylase precursor (gb AAD26634.1)
Up-regulated				
Metabolism				
At1g29900	1.26	1.18	0.60	Carbamoyl phosphate synthetase, large chain (carB)
At4g24620	1.27	1.17	0.70	Glucose-6-phosphate isomerase
At3g11670	1.28	0.98	0.67	Digalactosyldiacylglycerol synthase
At2g43100	**1.34**	**0.96**	**0.81**	**3-Isopropylmalate dehydratase, small subunit**
At1g01090	1.41	1.08	0.77	Pyruvate dehydrogenase E1, alpha subunit
At1g24280	1.53	1.03	0.75	Glucose-6-phosphate 1-dehydrogenase
At5g16290	1.74	1.15	0.72	Acetolactate synthase-like protein
At1g76490	1.81	0.86	1.77	Hydroxymethylglutaryl-CoA reductase (AA 1–592)
Photosynthesis				
At3g16140	1.26	0.92	0.67	PsaH1
At5g66190	1.27	1.25	0.62	PetH2; FNR; ferredoxin-NADP+ reductase
At5g66570	1.30	1.17	1.60	PsbO1
At3g08940	**1.49**	**0.92**	**0.84**	**Lhcb4.2 (CP29)**
At1g79040	1.53	0.91	1.66	PsbR
At1g15820	1.61	0.80	0.70	Lhcb6 (CP24)

TABLE I—*continued*

ATG[a]	Ratio[b] PSII/PSI	Ratio[b] PSII_DCMU/PSI	Ratio[b] PSI_DCMU/PSI	Description[c]
At1g31330	**1.71**	**0.91**	**1.17**	**PsaF**
At4g29670	1.89	0.82	1.58	Thioredoxin-like protein
Protein modification and fate				
At4g20740	1.30	1.11	0.78	Similarity to CRP1
At5g42390	**1.31**	**1.14**	**0.81**	**SPP/CPE**
Protein phosphorylation				
At5g25930	1.47	1.24	1.78	Receptor-like protein kinase-like
Protein synthesis				
At4g17300	**1.28**	**1.14**	**0.83**	**Asparagine-tRNA ligase**
Secondary metabolism				
At4g20230	1.46	1.18	1.36	Terpene cyclase-like protein
At5g38120	1.49	1.22	1.49	4-Coumarate-CoA ligase-like protein
At4g32540	**1.56**	**1.22**	**0.93**	**Dimethylaniline monooxygenase-like protein**
Stress response				
At4g11230	**1.36**	**1.12**	**0.89**	**Respiratory burst oxidase homolog F-like protein**
Transport				
At4g36520	1.30	1.15	0.77	Trichohyalin-like protein
At1g80830	**1.38**	**1.23**	**0.93**	**Metal ion transporter**
S-metabolism				
At5g27380	**1.26**	**1.20**	**0.96**	**Glutathione synthetase gsh2**

[a] Accession number.
[b] Expression data under the respective test condition relative to the expression data under PSI-light.
[c] Those genes among the classified 286 genes that have a clear functional assignment have been listed according to their down- or up-regulation under PSII light in comparison to PSI light. Genes are grouped into functional categories and listed according to their degree of regulation. Genes matching conditions of "ideal" redox regulation are given in bold letters.

TABLE II
Thiol group content and redox state of glutathione in differentially acclimated Arabidopsis seedlings

Growth light regime	Cysteine[a]	Glutathione	Reduced glutathione
	pmol/mg		*%*[b]
PSI	10.7 ± 0.7	319.5 ± 33.9	90.0 ± 2.4
PSI–II	10.8 ± 2.1	344.6 ± 65.0	87.3 ± 3.6
PSII	12.4 ± 1.2	390.2 ± 18.8	92.0 ± 1.5
PSII–I	10.0 ± 2.2	322.2 ± 73.1	88.7 ± 1.7

[a] Each value represents the average of four independent samples based on fresh weight, and S.D. is given.
[b] "%" refers to the proportion of reduced glutathione of total glutathione content.

the plastid-encoded RNA polymerase (PEP) (64). This suggests the existence of specific regulatory protein factors that might mediate the redox signal to the RNA polymerase. It is interesting to note that components for the PEP complex are found in the group of redox-regulated genes (see below). Furthermore, our primer extension studies identified a not yet described *psaA* 5′-end; however, this does not provide hints on putative redox-responsive *cis*-elements because the technique does not distinguish between transcript initiation and processing. *In vitro* DNA-protein interaction studies in spinach suggest that the *psaA* promoter may contain additional important regulatory elements, *i.e.* a so-called region D (65). Transcript initiation at this promoter therefore may play a key role during light quality acclimation in *Arabidopsis*. Experiments are in progress to characterize this regulation in more detail.

LTR in Photoreceptor and Chloroplast-to-Nucleus Signaling Mutants—Our PSI light source contains wavelengths over 700 nm, whereas the PSII light does not, resulting in different red/far red ratios that might affect the intracellular ratio of the phytochrome P_r and P_{fr} forms. However, because the LTR is present in all photoreceptor mutants tested, we conclude that the acclimatory response operates either independently from or above the photoreceptor signaling network. The observation that the LTR is only partially functional in the *phyA*/*phyB* mutant is most probably caused by pleiotropic side effects, because the double mutant exhibits severe developmental effects that may interfere with the LTR even if the general photosynthetic performance does not seem to be disturbed. The reversibility of the LTR within the single mutants provides a strong argument that the LTR is regulated without the signaling avenues of *phyA* or *phyB*. Both the PSI and the PSII light do not contain blue or UV-light, which is consistent with the observation that the LTR is not mediated by cryptochromes. The observed weaker response in the *cry2-1* mutant after a PSII–I light shift (Fig. 3*A*) must therefore be caused by a developmental side effect in this mutant. These data do not exclude interactions between redox and photoreceptor signaling networks, especially because many more genes are light- than redox-regulated, however, for the LTR, this appears to be meaningless. *Arabidopsis* photoreceptor mutants have also been used to test the involvement of photoreceptors in photosynthetic acclimation responses to high light (66, 67). In these studies the photoreceptor mutants acclimated to shifts in light intensity in a wild type-like manner. Although acclimations to light quality or light quantity involve different responses (1, 63), they all function in the absence of photoreceptors underlining the importance of photosynthetic acclimation in the response to environmental changes.

In the chloroplast-to-nucleus signaling mutants we also detected clear responses to the PSI and PSII light, indicating that the LTR operates independently of the lesions in these mutants. Only *cue1-6* lacks a significant LTR after a PSI–II light shift (as *phyA*/*phyB)*. The lack of the phosphoenol pyruvate carrier in *cue1-6*, however, has a strong impact on the energy metabolism of the mutant, and adult plants exhibit a reticular phenotype (58). Similar to the *phyA*/*phyB* double mutant, these developmental lesions might affect the LTR. None of the mutant lines investigated here lack the LTR completely except *hcf109*, which is the only mutant with defects in photosynthesis. The observation that in *phyA*/*phyB*, *cue1-6*, and *cry2-1* only one response is affected while the other is not could be a hint that reduction and oxidation signals can be separated and may operate via different pathways. It is interesting to note that in *cue* mutants a connection between phytochrome and plastid regulation of nuclear gene expression has been observed (68), although a connection between photosynthetic redox signals and other plastid retrograde signals or photoreceptors was not found here.

Impact of Light Quality on the Nuclear Chloroplast Transcriptome—The major goal of our array study was to determine the global impact of light quality and photosynthetic redox signals on the expression of nuclear genes for chloroplast proteins to assess the importance of such signals for higher plants. Light quality affects over 2000 genes encoding not only photosynthesis but also many other structural and functional components. Around 15% of these genes appear to be regulated by redox signals suggesting that many genes among the 2000 may be secondary or tertiary targets that are affected through the long term impact of redox signals on the overall cellular signaling network and/or the action of other light perceiving systems. Many genes exhibit relatively small changes in their expression. This can be best explained by the fact that the expression profiles were determined at the end of the acclimatory response when a new expression equilibrium has been established. Genes transiently affected only for a short time after a light switch or an inhibitor application might be not detected by this approach and will be identified by further, more detailed, studies.

The expression profile after acclimation to a reduction signal exhibits similar numbers of up- and down-regulated genes (Fig. 5). In a hierarchical cluster analysis of expression profiles in 35 different physiological situations or mutants with this macroarray, our profile was found to be the most prominent representative of the so-called class 2 profiles, which are characterized by balanced expression changes (43). Class 1 profiles showed mainly up-regulated and class 3 profiles mainly down-regulated genes. Among the latter two classes the profiles of the *gun* (class 1) and *cue* mutants (class 3) were found. The different profile clustering is an independent confirmation that in these mutants gene expression regulation appears to be totally different from that observed under our conditions. This again argues for the independence of light quality-induced redox signals from the plastid signaling pathways, which are defective in the *gun* and *cue* mutants.

It is difficult to discuss complex results such as transcript profiles on the level of individual genes, however, the study uncovered many interesting genes responding to redox signals. Some of them that are of special interest for the LTR and its regulation are highlighted in the following. We found several groups of redox-regulated genes encoding products with related functions, including those for photosynthesis (Table I, up-regulated). All affected genes encoding components of the photosynthetic machinery were found to be up-regulated by a reduction signal. A prominent representative is the *PsaF* gene, which exhibits essentially the same expression profile as obtained earlier with transgenic tobacco lines containing a *PsaF*-promoter::*uidA* construct (14), demonstrating the reproducibility of the expression data. We also found a thioredoxin-like protein that is of interest because thioredoxins regulate many processes in chloroplasts such as light induction of Calvin cycle enzymes or translation initiation of *psbA* (69). In general, up-regulation occurred for both PSII and PSI genes suggesting that the stoichiometric adjustment of the nuclear encoded components includes additional regulatory steps at other levels of expression and/or complex assembly (see below). This might also be the reason why we did not find all nuclear photosynthesis genes to be regulated in this array.

Metabolic genes represent the most prominent group among the redox-regulated genes identified here. Most encode components involved in amino acid and nucleotide metabolism and are regulated in the opposite way to photosynthesis genes. Amino acids and nucleotides are central molecules in many biosynthetic pathways demonstrating that the acclimation response is not restricted to photosynthesis but has also a deep impact on the metabolism of a plant. A metabolic gene of special interest here is the succinyl-CoA-ligase, which produces the precursor molecule for aminolevulinic acid, the entry substance for chlorophyll biosynthesis, a process that is clearly affected during the LTR (Fig. 1*C*). In addition, we found the digalactosyldiacylglycerol synthase, which produces the major lipid of thylakoid membranes (70). The LTR involves major re-arrangements of the thylakoid membrane system in chloroplasts.[2] Because of these results we have started further studies to investigate the LTR effects on plant metabolism in more detail.

A further striking observation is the regulatory impact on components of the chloroplast PEP enzyme (*rpoB*, sigma-like factor, *SigA* binding factor; Table I, transcription, down-regulated), which is responsible for the redox regulation at the *psbA* and *psaAB* promoters (Fig. 2). The *rpoB* gene is plastid-localized, encodes the catalytic β-subunit of PEP, and is transcribed by the nuclear encoded RNA polymerase (64). This suggests a redox regulation of nuclear encoded RNA polymerase activity. Interestingly the paralogue nuclear encoded RNA polymerase gene, which encodes the mitochondrial nuclear encoded RNA polymerase, appears to be redox-regulated in its expression like ARR9, ARR12, and GBF4, transcriptional regulators of nuclear transcription, as well as the sigma-like-factor and *SigA* binding factor, transcriptional regulators of chloroplast transcription. This suggests a complex signaling network controlling in parallel the expression of the different components of the plastid gene transcription machinery in the nucleus and in the organelle. Furthermore, regulation of the PEP enzyme has been shown *in vitro* to be under phosphorylation control via the plastid transcription kinase, which itself is under control of glutathione redox state (16). Our results do not indicate major differences in the glutathione redox state under the various light conditions thus supporting the idea of several different redox control pathways in chloroplast transcription (60, 71), depending on environmental conditions as to be expected for different acclimation responses under low or high light (see above).

The photosystems are multiprotein complexes, which have to be assembled in a highly coordinated manner. Several chaperones and assembly proteins were identified as being redox-regulated (Table I, up-regulated, protein modification, and fate). Important in this context are ALB3 and SRP54, two proteins of the SRP complex in thylakoid membranes that are responsible for the import of light-harvesting proteins into the thylakoid membrane (72, 73).

Of special interest is the observation that several genes for enzymes involved in sulfur and glutathione metabolism (Table I, *S*-metabolism) together with genes for products involved in various stress responses (choline monooxygenase, lypoxygenase, and respiratory burst oxygenase) are found to be redox-regulated. It is possible that these changes in glutathione genes are responsible for the relatively stable glutathione redox state found here. It is also possible that these genes are regulated together with stress genes (see above) in a kind of overlap reaction between photosynthetic redox signals and other environmentally induced stress signals such as cold (indicated by choline monooxygenase (74)) or pathogen attack (indicated by lypoxygenase, respiratory burst oxygenase (75)), which are also mediated by redox signals. It is well known that interactions between photosynthesis, temperature, or pathogen attack exist and that redox signals of various origin play a central role in this scenario. Antioxidant molecules such as glutathione are involved in all these processes indicating that multiple connections between the responses to the different environmental stress situations exist (76). The dominant regulatory signals

controlling the LTR appear to come from the electron transport chain, because the glutathione redox state remained relatively stable under all conditions. Because the redox state of ascorbate is tightly coupled to that of glutathione (31), we expect that the antioxidant network remains in homeostasis during the LTR, which makes it very unlikely that in our light quality system reactive oxygen species play a significant regulatory role.

To our knowledge this is the first report describing the effects of DCMU on gene expression in a higher plant using an array approach. A similar study has been performed so far only with a whole genome array of *Synechocystis* (77). By the use of DCMU and 2,5-dibromo-3-methyl-6-isopropyl-*p*-benzoquinone, 140 genes have been reported to be affected by redox signals from the electron transport chain, which is in the same order of magnitude as in our experiment. However, Hihara *et al.* (65) concluded that the redox regulation of photosynthesis genes in *Synechocystis* might be totally different from that in algae and plants. A gene-by-gene comparison between both studies does not provide much useful information, even if we consider that in our array the eukaryotic complement of the cyanobacterial genome is present, because the physiological conditions used in both studies are very different.

DCMU also affected photosynthetic electron flow in plants grown continuously under PSI light indicating that these plants perform linear electron transport. The expression profile of these plants, however, is different from that of PSI–II plants treated with DCMU suggesting that possibly more redox-regulated genes exist than described here. The combined action of DCMU and PSI light on photosynthetic electron flow is difficult to understand to date and requires further detailed analyses; therefore, we described only those genes as redox-regulated that allow us to conclude unambiguously on such a regulation. Data from different studies suggest the existence of several yet unknown redox signals originating from the electron transport chain, including PSII (30, 78, 79). Furthermore, any change in linear electron flow will affect the redox state of components downstream of PSI such as thioredoxin, which in turn will affect the efficiency of the Calvin cycle (69). Whether such signals influence gene expression events in our experimental system is currently under investigation.

Our study indicates that photosynthetic redox signals play an important role in the intracellular signaling network. The photosynthetic redox signals contribute essential information about the light environment in addition to cytosolic photoreceptors thus significantly expanding the ability of plants to sense environmental cues. It appears that this information is transferred from the organelle to the nucleus by mechanisms that differ from other chloroplast-to-nucleus signaling avenues and without the help of photoreceptor-mediated signaling.

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Additions and Corrections

Vol. 280 (2005) 5318–5328

Retrograde plastid redox signals in the expression of nuclear genes for chloroplast proteins of *Arabidopsis thaliana*.

Vidal Fey, Raik Wagner, Katharina Bräutigam, Markus Wirtz, Rüdiger Hell, Angela Dietzmann, Dario Leister, Ralf Oelmüller, and Thomas Pfannschmidt

Page 5321, Fig. 2: The positions of the *psaA* transcripts given in Fig. 2 are incorrect. The correct figure is shown below. As a result, a sentence appearing in the left column, lines 29–31 of the same page, should read as follows: "For *psaA* we found two prominent 5′-ends in a distance of 189 and 76 bases upstream of the translation initiation codon."

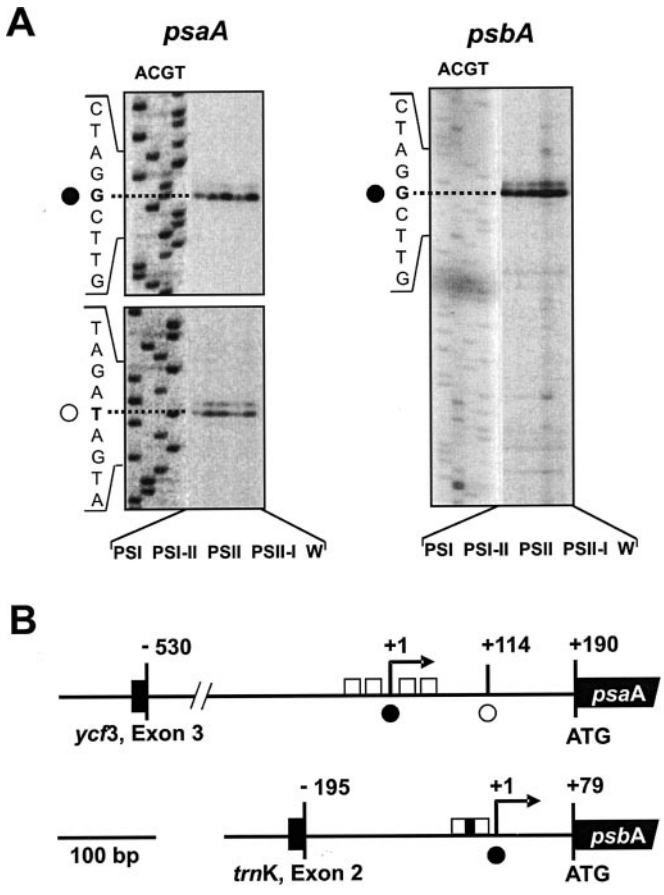

FIG. 2

We suggest that subscribers photocopy these corrections and insert the photocopies at the appropriate places where the article to be corrected originally appeared. Authors are urged to introduce these corrections into any reprints they distribute. Secondary (abstract) services are urged to carry notice of these corrections as prominently as they carried the original abstracts.

III

Fey, V., **Wagner, R.**, Bräutigam, K., Pfannschmidt, T. **(2005)**. "Photosynthetic redox control of nuclear gene expression." Journal of Experimental Botany 56(416): 1491-1498.

In diesem Artikel werden die Erkenntnisse der letzten Jahre zum Gebiet der photosynthetischen Expressionsregulation nukleärer Gene zusammengefasst. Dabei wird besonderes Augenmerk auf die Regulation solcher Genprodukte gelegt, die in die Plastiden importiert werden müssen. Es wird diskutiert, welche photosynthetischen Parameter bereits dafür bekannt sind, nukleäre Genexpression zu verändern. In einem Modell werden Plastidensignale und Signale zytosolischer Photorezeptoren dargestellt und es wird diskutiert, wie diese interagieren können.

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FOCUS PAPER

Photosynthetic redox control of nuclear gene expression

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Abstract

Chloroplasts contain 3000–4000 different proteins but only a small subset of them is encoded in the plastid genome while the majority is encoded in the nucleus. Expression of these genes therefore requires a high degree of co-ordination between nucleus and chloroplast. This is achieved by a bilateral information exchange between both compartments including nucleus-to-plastid (anterograde) and plastid-to-nucleus (retrograde) signals. The latter represent a functional feedback control which couples the expression of nuclear encoded plastid proteins to the actual functional state of the organelle. The efficiency of photosynthesis is a very important parameter in this context since it is influenced by many environmental conditions and therefore represents a sensor for the residing environment. Components of the photosynthetic electron transport chain exhibit significant changes in their reduction/oxidation (redox) state depending on the photosynthetic electron flow and therefore serve as signalling parameters which report environmental influences on photosynthesis. Such redox signals control chloroplast and nuclear gene expression events and play an important role in the co-ordination of both genetic compartments. It is discussed here which photosynthetic parameters are known to control nuclear gene expression, how these signals are transduced toward the nucleus, and how they interact with other plastid retrograde signals and cytosolic light perception systems.

Key words: Crosstalk, nuclear gene expression, photoreceptor, photosynthesis, retrogade signalling, redox regulation, signal transduction networks.

Introduction

During evolution, photosynthetic organisms developed a great number of molecular mechanisms which enable them to acclimate the photosynthetic process to a fluctuating environment (Aro and Andersson, 2001; Blankenship, 2002). Changes in temperature, nutrient and water supply, and particularly in incident light parameters affect photosynthetic electron transport and can dramatically reduce its efficiency. Acclimation responses maintain or restore the photosynthetic electron flux under adverse conditions and, by such means, help to keep the net energy fixation as high as possible. This can be observed in organisms with a short generation cycle such as cyanobacteria and unicellular algae as well as in long-living multicellular organisms such as plants. The biological reason for this phenomenon may be different but the goal behind it is the same. Single-celled organisms have to respond very dynamically to rapid changes in their environment because otherwise growth and progeny are at risk. Plants may exhibit a higher tolerance to rapid environmental changes because of their larger body, which provides a higher storage capacity for most nutrients or energy equivalents, but, because of their sessile life style, plants have to deal with the longer-lasting conditions of the area where they grow in order to allow sufficient growth and success in seed production. Acclimation of photosynthesis is a central strategy in this context as this process provides the energy for all cellular processes.

In eukaryotic organisms, photosynthesis takes place in a specialized compartment, the chloroplasts. This organelle contains a complex membrane system, the thylakoids, in which the photosynthetic machinery is located. It consists of several multi-subunit protein complexes (photosystem I and II (PSI and PSII), the cytochrome b_6f complex (cytb_6f) and the ATPase) which are comprised of components

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Abbreviations: Chl, chlorophyll; cytb_6f, cytochrome b_6f complex; DBMIB, 2,5 dibromo-3-methyl-6-isopropyl-*p*-benzoquinone; DCMU, 3-(3′,4′-dichlorophenyl)-1,1′-dimethylurea; *gun*, *genome uncoupled*; *laf*, *long after far red light*; *Lhcb*, light-harvesting complex chlorophyll binding protein of photosystem II; MgProtoIX, magnesium protoporphyrin IX; *PetE*, plastocyanin; *PetF*, ferredoxin-NADP-oxidoreductase or FNR; PSI, photosystem I; PSII, photosystem II; *PsaD*, subunit II of PSI; *PsaF*, subunit III of PSI; PQ, plastoquinone; ROS, reactive oxygen species.

encoded in the nucleus as well as in the plastome, the organelle-own genome (Sugiura, 1995). For a eukaryotic cell this generates several problems in the establishment and reconstruction of the photosynthetic apparatus during development and environmental acclimation. Nuclear-encoded components must be expressed in the nucleus, translated in the cytosol, and subsequently imported into the chloroplast (Jarvis and Soll, 2002), while the plastid-encoded components are expressed in the organelle by a plastid-own expression machinery (Link, 1996; Hess and Börner, 1999; Liere and Maliga, 2001). The resulting products then have to be assembled into functional protein complexes. Since photosynthetic plant cells contain up to 100 plastids and each plastid contains up to 100 copies of the chloroplast genome this can generate up to a 10 000-fold excess of plastid over nuclear gene copies. Both the different coding location and the varying gene copy number require a high degree of co-ordination in the expression of genes encoding components of the photosynthetic machinery. This co-ordination is achieved by a continual exchange of information between nucleus and plastids. Early studies suggested a complete control of the nucleus in this event, but studies from the last 20 years have provided accumulating evidence that the plastids also send important signals to the nucleus about its functional state, which help to express genes for chloroplast proteins in a co-ordinated manner. Nowadays, it is widely accepted that the co-ordination of plastid protein complex formation involves bidirectional signalling pathways, including signals from the nucleus to the plastids (anterograde signals) and vice versa (retrograde signals) (Taylor, 1989; Mayfield, 1990; Susek and Chory, 1992; Goldschmidt-Clermont, 1998; Rodermel, 2001; Gray *et al.*, 2003).

Retrograde signals from chloroplasts affecting nuclear gene expression

In the 1980s, studies were published reporting that seedlings with carotenoid deficiencies (induced either by mutation or by treatment with the phytoene desaturase inhibitor norflurazon) exhibit down-regulation of nuclear photosynthesis genes (Mayfield and Taylor, 1984; Batschauer *et al.*, 1986; Oelmüller *et al.*, 1986; Mayfield and Taylor, 1987). Carotenoids are important components of stress-compensating processes which can scavenge triplet excited-state chlorophyll or singlet oxygen and, by this means protect the organelle from photo-oxidative damage (Oelmüller, 1989). Chloroplasts without these scavengers become photo-oxidized resulting in non-functional, pale organelles lacking the typical thylakoid membrane system, photosynthetic pigments, and many photosynthesis-related proteins. This demonstrated that the expression of nuclear PS genes is coupled to a functional photosynthetic process. Other studies especially with the unicellular alga *Chlamydomonas rheinhardtii* revealed that intermediates of chlorophyll (Chl) biosynthesis, i.e. magnesium protoporphyrin IX (MgProtoIX) might be potent regulators of nuclear gene expression events. Initial studies reported repression of nuclear photosynthesis genes when Chl biosynthesis was specifically inhibited, resulting in an accumulation of precursor molecules such as MgProtoIX (Johanningmeier and Howell, 1984; Johanningmeier, 1988). Furthermore, it could be shown that MgProtoIX was able to induce nuclear-encoded cytosolic and plastid heat-shock proteins (Kropat *et al.*, 1997, 2000). Both carotenoid and chlorophyll biosynthesis occur in the plastids. The observed involvement of these components or pathways therefore led to the postulation of a so-called 'plastid-derived factor' which controls the expression of nuclear-encoded PS and non-PS genes (Oelmüller, 1989) (Fig. 1). In addition, inhibitor experiments blocking plastid gene expression revealed that nuclear photosynthesis gene expression in the first days of seedling development is also dependent on plastid transcription and translation (Pfannschmidt and Link, 1997; Sullivan and Gray, 2002) suggesting that there also exists a light-independent signal which must involve a plastid gene product (Fig. 1).

The strong involvement of Chl biosynthesis in retrograde signalling was further confirmed by several studies in the higher plant *Arabidopsis thaliana*. So-called *genome uncoupled* (*gun*) mutants exhibit proper expression of a *Lhcb* promoter::*uid*A reporter gene construct, despite a bleaching of the seedlings by norflurazon treatment which normally would suppress *Lhcb* expression (Susek *et al.*, 1993). All

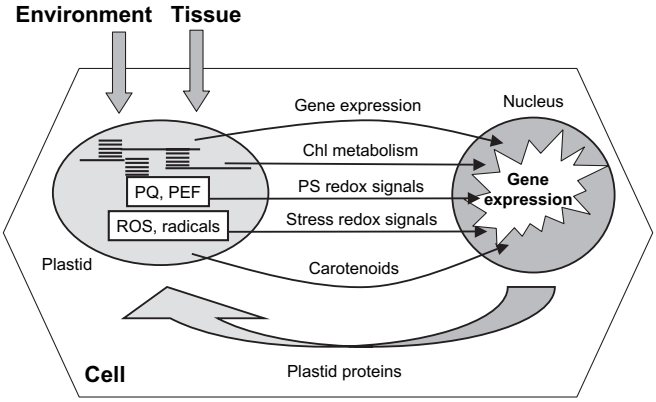

Fig. 1. Retrograde plastid signals known to affect nuclear gene expression. A cell containing a plastid and a nucleus is schematically drawn. Plastid function and development is influenced by environmental signals and, in the case of multicellular organisms, by the respective tissue context in which the cell is present. The functional and/or developmental state of the plastid(s) is reported to the nucleus by various signals (see text) where they have to be integrated. Expression of genes encoding plastid proteins is then regulated according to the needs of the organelle(s) and the resulting gene products are transported into the plastid. For simplicity, the retrograde signals are drawn as independent arrows, however, it must be considered that these signals possibly interact or act via the same or related pathways.

gun alleles which could be identified until now encode important enzymes or enzyme subunits of the tetrapyrrol biosynthetic pathway (Strand, 2004). Interestingly, other knock-out mutants in this pathway also revealed a *gun* phenotype in the presence of norflurazon, but only when the lesion was upstream of MgProtoIX (Strand *et al.*, 2003; Strand, 2004) giving further strong support of the involvement of MgProtoIX in plastid-to-nucleus signalling. Another *Arabidopsis* mutant, the *long after far red 6* (*laf6*) mutant was found to be deficient in a novel ABC transporter protein (Moller *et al.*, 2001) and to exhibit high amounts of cytosolic protoporphyrin IX. Inhibitor experiments suggested that this transporter is responsible for the import of protoporphyrin IX into the stroma which cannot occur in the *laf6* mutant which, in turn, resulted in a repression of nuclear photosynthesis genes which is consistent with the above-mentioned studies.

All relevant reports and findings in this field of research cannot be discussed in this review but, from the given examples, it becomes very obvious that a functional photosynthetic process plays an important role in plastid-to-nucleus signalling. Many of the experiments discussed above use very artificial or destructive approaches to analyse the plastid retrograde signals. Therefore the focus here is on experiments done under more physiological conditions which show that the photosynthetic process itself is very important in retrograde signalling. The redox state of electron transport chain components as well as photosynthetic products like sugars and unavoidable photosynthetic by-products such as reactive oxygen species (ROS) were found in numerous studies to be involved in plastid-to-nucleus signalling (Brown *et al.*, 2001; Rodermel, 2001; Dietz, 2003; Pfannschmidt, 2003) and, therefore, represent a novel class of plastid signals (Pfannschmidt *et al.*, 2003). However, there must be a clear distinction between redox signals which are generated in the photosynthetic electron transport chain due to fluctuating environmental conditions within the physiological range and those which are produced under extreme conditions resulting in stress reactions (Fig. 1) (Apel and Hirt, 2004; Wagner *et al.*, 2004). Many of these stress reactions are covered by other excellent reviews in this issue. Here, the focus is on retrograde signals originating directly from fluctuations in the redox state of photosynthetic electron transport chain components, i.e. the redox state of the plastoquinone (PQ) pool which transfers electrons from PSII to the cytb_6f complex and, therefore, represents an ideal sensor for the efficiency of photosynthetic electron flow.

Photosynthetic control of the nuclear *Lhcb* genes: the classical example

The chlorophyll binding proteins of the light-harvesting complex of photosystem II are encoded by the large nuclear *Lhcb* gene family (formerly known as chlorophyll *a/b* binding proteins, *cab*). They represent the genes most studied in the context of plastid-to-nucleus signalling and are shown to respond to many of the retrograde signals (compare studies above). Therefore, it is not surprising that in initial reports this gene group was also used to study a possible influence of photosynthetic signals on nuclear gene expression. In the unicellular alga *Dunaliella tertiolecta* the expression of *Lhcb* genes was shown to be stimulated by an oxidized PQ pool which was established by a switch of the cultures from high to low light intensities. This result was supported by experiments with the site-specific electron transport inhibitors DCMU and DBMIB (Escoubas *et al.*, 1995) (Fig. 2). In a physiologically different approach using the related alga *Dunaliella salina* it was investigated how this alga responds to changes in incident light quantity under a varying temperature environment (Maxwell *et al.*, 1995). This experiment took into account that high light effects can be mimicked by low light intensities under low temperature. A decrease in temperature induces a down-regulation of enzymatic reactions of the Calvin cycle, which subsequently leads to an increase in excitation pressure of PSII as it occurs under high light

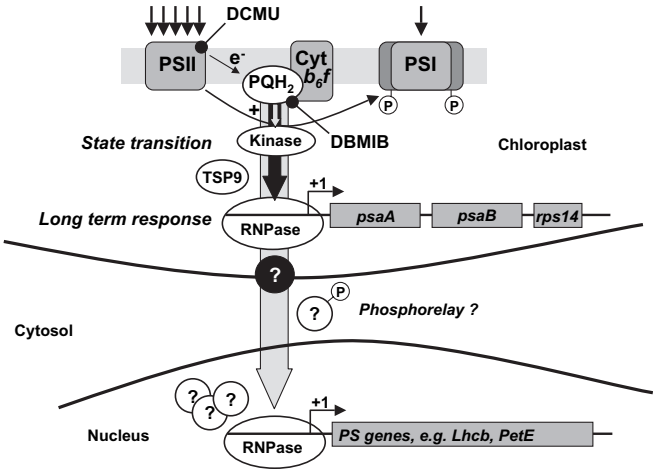

Fig. 2. Events and targets regulated by the redox state of the plastoquinone pool. The photosynthetic electron transport chain is drawn schematically. Arrows on top of the photosystems indicate favoured excitation of PSII resulting in a reduced PQ pool (PQH_2). Sites of action of electron transport inhibitors DCMU (preventing reduction of PQ) and DBMIB (preventing oxidation of PQ) are indicated by black dots. The reduction of PQ activates the LHCII kinase (kinase) which, in turn, phosphorylates (P) the mobile part of LHCII (dark grey blocks). This migrates to PSI, enlarging its antenna cross-section (*state transition*). The same signal positively affects the transcription of the *psaAB* operon through the plastid RNA polymerase (RNPase) controlling photosystem stoichiometry adjustment (*long term response*). The signal is also transported over the chloroplast double membrane by an unknown mechanism (question mark), transported through the cytosol into the nucleus where it affects the expression of *Lhcb* and other nuclear-encoded plastid proteins. Proteins possibly involved in the signal transduction are given as light grey circles or ovals, affected genes as bars. The arrows with +1 mark transcription start points. For details see text.

conditions at moderate temperature (Huner *et al.*, 1998). Taken together, these results suggest that the redox state of the PQ pool controls the transcription of nuclear *Lhcb* genes, i.e. under high PSII excitation pressure (PQ reduced) *Lhcb* expression is repressed to avoid absorption of excess light energy. This suggests the existence of a regulatory pathway originating from the PQ pool and leading to an increase in the amount of light-harvesting complexes and, subsequently, the quantum yield of the PS apparatus of *Dunaliella* species under low light or PQ oxidizing conditions. Studies on *Lemna perpusilla* revealed that this mechanism is not restricted to algae but also occurs in higher plants (Yang *et al.*, 2001). In this work, a cytb_6f-deficient *Lemna* mutant was characterized and compared with the wild type. The PQ pool was found to be reduced in the mutant even under low light conditions because no plastoquinol oxidation could occur. In parallel, the LHCII amount was found always to be lower in the mutant than in the wild type. This effect could be abolished by blocking PQ reduction. Therefore, it was concluded that the mutant is locked in a high-light acclimated state which represses LHCII expression via the reduced PQ pool, indicating that the PQ pool is the sensor for the high-light illumination. However, in pumpkin, it was also shown that, under high light intensities, a second loop of redox regulation may be active which is responsible for LHCII de-phosphorylation via repression of the LHCII kinase through the action of a reduced ferredoxin thioredoxin system (Rintamaki *et al.*, 2000). This suggests a more complex high light redox regulation in higher plants than in algae.

However, while the results concerning the existence of this mechanism and its functional role appear relatively clear, the signal transduction is still not understood. The PQ pool is known to regulate both short- and long-term acclimatory responses (Allen and Pfannschmidt, 2000). It is yet not clear if these signal pathways represent two parallel branches with the same origin or a hierarchically organized signalling cascade. The present results support the latter idea (Allen and Pfannschmidt, 2000; Pursiheimo *et al.*, 2001). In a working model (valid for low light intensities) (Fig. 2) the PQ pool controls phosphorylation of the mobile part of LHCII through activation of the LHCII kinase upon reducing conditions. This redirects the antenna proteins (via phosphorylation) from PSII to PSI and helps to redistribute excitation energy between the two photosystems. At the same time the efficiency of this response regulates the strength of the redox signal of the PQ pool which is directed toward the plastid gene expression machinery. Upon prolonged reduction it activates transcription of the plastid *psaAB* operon encoding the P700 apoproteins of PSII (Pfannschmidt *et al.*, 1999*a*; Pfannschmidt, 2003) which triggers a long-term readjustment of photosystem stoichiometry. This has the same, but a longer lasting, effect as the short-term response in which only the antenna structure is modified. How the signal from the thylakoid membrane is transported to the RNA polymerase is also under investigation. A possible candidate as signal transducer is a small 9 kDa protein called TSP9 which is associated with PSII and which is partially released from the complex under reducing conditions (Carlberg *et al.*, 2003). At its C-terminal end this protein contains a basic region which possibly acts as a DNA-binding domain. However, both DNA-binding activity and a functional connection to long-term acclimation responses still have to be elucidated.

As outlined in the introduction, a long-term response must include the control of nuclear-encoded plastid proteins. A respective regulation via the redox state of PQ has been demonstrated for *Lhcb* in winter rye (Pursiheimo *et al.*, 2001) and for *PetE* (encoding plastocyanin) in tobacco (Pfannschmidt *et al.*, 2001). How the redox signal from PQ passes the chloroplast envelope is completely unknown (Fig. 2, black circle with question mark), however, for its transduction through the cytosol into the nucleus experimental data exist which suggest a possible phosphorylation cascade. In transgenic tobacco, a *PsaF* promoter::*uid*A reporter gene construct can be induced by cytosolic kinase activities even in the absence of functional plastids (Chandok *et al.*, 2001). Again, in *Dunaliella tertiolecta*, initial results suggest that a cytosolic protein becomes phosphorylated when PQ is oxidized and which then possibly binds to the *Lhcb* promoter (Escoubas *et al.*, 1995). It therefore may represent a functional link between the redox state of the PQ pool in the chloroplast and *Lhcb* transcription in the nucleus (Fig. 2). However, its identity and precise role in the signal transduction chain remains to be elucidated. A further recent study identified several different protein–DNA complexes in the *Lhcb1* promoter of *Dunaliella tertiolecta* (Chen *et al.*, 2004), suggesting that more than one protein is involved in this regulation (Fig. 2, orange circles with question marks). This study also revealed that, beside the redox state of PQ, the *trans*-thylakoid pH gradient is responsible for *Lhcb* regulation suggesting interaction of two (or more?) photosynthetic signals in this regulation mechanism. Therefore, the working model presented provides only a rough picture of what is really going on.

Studying *Lhcb* gene expression gave the first results of how thylakoid electron transport and other plastid signals affect nuclear gene expression. The high responsiveness of this gene family to plastid signals made it a useful tool with which to perform the initial experiments. However, it is now necessary to go one step further. There are many more nuclear-encoded plastid proteins which might be affected by plastid signals. In addition, several lines of evidence suggest that there are more redox signals than that from the PQ pool (see above and below). To understand the redox signalling network originating from photosynthesis in more detail, more genes under several, different, physiological conditions have to be analysed.

Photosynthetic control of nuclear genes encoding chloroplast proteins during light-quality acclimation

Under many conditions, for example, in the canopies of trees or in dense plant populations, strong light quality gradients occur which often result in uneven excitation of the two photosystems. Such excitation imbalances reduce photosynthetic efficiency and are counteracted in the long-term by a photosystem stoichiometry adjustment (Melis, 1991; Allen, 1995; Fujita, 1997; Pfannschmidt, 2003). An artificial light quality system has been established which mimics such light quality gradients by a favoured excitation of PSII (PSII-light) or PSI (PSI-light). By shifting plants between such light sources it was possible to induce a more reduced (shift from PSI- to PSII-light) or oxidized (shift from PSII- to PSI-light) state of the photosynthetic electron transport chain. This gave a very useful experimental design with which to study redox effects of photosynthetic electron transport on nuclear gene expression. Comparable systems have been used to elucidate the impact of photosynthetic electron transport on plastid gene expression in various higher plants (Glick *et al.*, 1986; Deng *et al.*, 1989; Kim *et al.*, 1993; Pfannschmidt *et al.*, 1999*a*, *b*; Tullberg *et al.*, 2000). Using transgenic tobacco lines harbouring several nuclear PSI gene promoter::*uid*A reporter gene constructs, the impact of photosynthetic redox signals was tested on the activity of the promoters for *PetE*, *PsaF*, *PsaD*, and *PetH* encoding plastocyanin, subunit III and II of PSI, and the ferredoxin-NADP-oxidoreductase (FNR), respectively (Pfannschmidt *et al.*, 2001). All these subunits are implicated in electron transport around PSI and the promoters have been found in earlier experiments to respond to light and a plastid signal (Kusnetsov *et al.*, 1996). The experiments revealed that the *PetE* promoter is controlled by a redox signal from the PQ pool, while *PsaD* and *PsaF* respond to a redox signal more downstream of PQ (with respect to the electron transport). *PetH* showed no reaction. In another study, experiments with an *Arabidopsis* cell culture also indicated an important role for photosynthetic redox signals in the regulation of *PetE*. However, from their results the authors concluded that thioredoxin or glutathione, rather than the PQ pool, was the origin of the signal (Oswald *et al.*, 2001). These conflicting results may be attributed to differences in the experimental set-up or the organisms used. Nevertheless, the authors' results with the transgenic lines clearly indicated that the light system was a useful tool with which to study the impact of photosynthetic redox signals on nuclear gene expression. In addition, the diverse responses led to the conclusion that light-dependent plastid signals are not always identical with photosynthetic redox signals and, furthermore, that several signals from the electron transport chain are sent. To get a more general impression of the impact of light-quality-induced redox signals on the expression of nuclear-encoded chloroplast proteins, a macroarray approach with *Arabidopsis* was set up in which it was tested how many of these genes respond to photosynthetic redox signals in this study's light system (Fey *et al.*, 2004). Western analyses confirmed that *Arabidopsis* shows the same long-term response, i.e. photosystem stoichiometry adjustment as observed before in mustard (Pfannschmidt *et al.*, 1999*a*), however, the stoichiometry changes were achieved by only changing the number of PSI while PSII remained constant. This indicates species-specific differences in the molecular realization of the long-term response, without affecting the basic principle which is a stoichiometry adjustment in favour of the respective rate-limiting photosystem. The macroarray used in the study contained 3292 GSTs covering a great proportion of the nuclear chloroplast transcriptome (Kurth *et al.*, 2002; Richly *et al.*, 2003). Comparing the expression profiles under different redox states of the electron transport chain (either induced by light quality shifts and/or DCMU treatment) 286 genes were identified which significantly respond to photosynthetic redox signals. Only 76 of these genes have an assigned function, while the great majority encodes unknown or putative products. This indicates an immense impact of photosynthesis on nuclear gene expression in this system. The affected genes were not restricted to photosynthesis and covered all major gene groups including those for gene expression, metabolism, and signal transduction. The largest group of affected genes were those encoding enzymes of amino acid, nucleotide, and energy metabolism suggesting that the array data reflect different metabolic situations of the plants under PSI- and PSII-light. A detailed investigation to understand the relationship of these gene expression changes with metabolic changes is currently in progress. Of special interest is the regulatory impact on subunits of the plastid-encoded RNA polymerase (PEP) (Liere and Maliga, 2001), including the catalytic subunit *RPOB* and the regulatory subunits sigma-like factor and *SigA*-binding factor, as well as effects on nuclear transcription factors (GBF4, ARR9 and 12) and the mitochondrion-located nuclear-encoded RNA polymerase. This suggests a network which controls and co-ordinates the gene expression machineries in all three genetic compartments. Although a constant redox state of the glutathione pool was found under all growth conditions, genes for glutathione metabolism and a few stress genes were detected by the array approach. This could be a hint that the signalling networks of photosynthetic redox control and environmental stresses overlap to a certain degree. In summary, this clearly demonstrates that redox signals from chloroplasts represent a complex signalling network that affects multiple genes. Analysis of single genes, therefore, may help only in understanding single facets of this network.

The great impact of redox signals on nuclear gene expression also implies a possible interaction with other

signalling pathways or networks. In particular, photoreceptor-mediated light responses and crosstalk with other retrograde signals are of interest since here, at least in part, over-lapping functions are likely to be expected. This question was addressed by testing the long-term response of various photoreceptor and retrograde signalling *Arabidopsis* mutants using the non-invasive Chl fluorescence parameter F_s/F_m (Pfannschmidt *et al.*, 2001; Sherameti *et al.*, 2002). Mutants deficient in phytochrome A and B as well as in cryptochrome 1 and 2 (Fey, Wagner *et al.*, 2004) were tested. They all exhibited wild-type-like responses indicating that these photoreceptors are not essential for photosystem stoichiometry adjustment. Mutants deficient in the products of the genes *gun1* (unknown product), *gun4* (encoding a substrate binding protein of the magnesium chelatase; Larkin *et al.*, 2003), *gun5* (encoding the ChlH subunit of the magnesium chelatase; Mochizuki *et al.*, 2001), and *cue1-6* (encoding the phospho*enol*pyruvate/phosphate translocator of the chloroplast envelope; Streatfield *et al.*, 1999) were also tested. Like the photoreceptor mutants they all showed a response comparable to the wild type. This led to the conclusion that the redox signals generated by light quality shifts induce the acclimatory responses without the help of the tested photoreceptors or retrograde signalling pathways (Fig. 3). Of course it cannot be excluded that such signalling cascades also affect redox-regulated genes, however, for the establishment of the long-term response, the decisive signals come from the photosynthetic electron transport chain. This is consistent with observations demonstrating that the acclimation of *Arabidopsis* to changes in light intensity is also functional in photoreceptor-deficient mutants (Walters *et al.*, 1999; Weston *et al.*, 2000). From the present data it appears that photosynthesis represents a major environmental sensor and regulator of respective cellular acclimatory responses. This significantly expands the range in which plants detect and react to the environment beside photoreceptor-mediated responses.

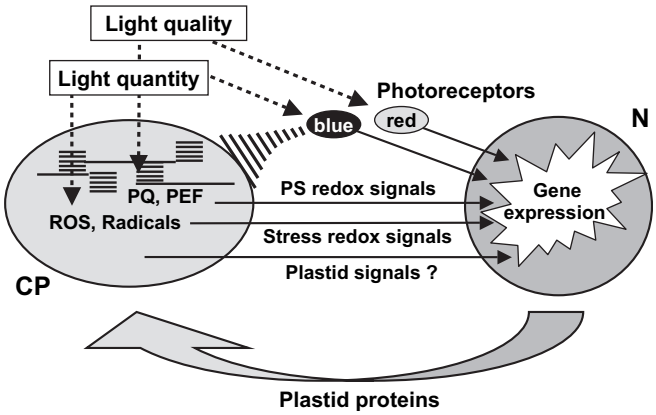

Fig. 3. Light quality effects on nuclear gene expression. The light environment is perceived by both cytosolic photoreceptors (black oval marked with 'blue' for blue light receptors, white oval marked with 'red' for phytochromes) and photosynthesis (thylakoid membrane system in the chloroplast (CP)). Light quality effects can be found only under low light conditions and induce variations in photosynthetic electron flow (PEF) or the redox state of plastoquinone (PQ). Light quantity also affects PEF and PQ, but high intensities can also lead to the generation of reactive oxygen species (ROS) or other radicals. Strong light also leads to chloroplast movements which are mediated by blue absorbing phototropins (indicated by extending black lines). Both systems report the respective condition by independent or not related pathways to the nucleus (N) where they affect gene expression. The involvement of other plastid signals in this event is not likely from the present data (see text). Photosynthetic redox signals therefore work in parallel to photoreceptor-mediated signals to induce synthesis of plastid proteins appropriate to the residing environment.

Perspectives

The role of photosynthetic redox signals in plant gene expression appears to become more and more prominent. The present data clearly indicate that a complex redox signalling network exists in plant cells which becomes even more complex when redox signals from stress responses and other cellular compartments such as mitochondria or peroxisomes were also considered. It will be fascinating to unravel the relationships between these signals as well as any possible crosstalk with other signalling networks. On the other hand, it is quite clear that this is just the beginning. Several important points in this field of research are still not understood, i.e. the transduction of photosynthetic redox signals from the thylakoid membrane toward the level of gene expression both in chloroplasts and the nucleus. Molecular reconstruction of these mechanisms will be a milestone in plant research which will facilitate an understanding of this whole area of research.

Acknowledgements

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IV

Bonardi, V., Pesaresi, P., Becker, T., Schleiff, E., **Wagner, R.**, Pfannschmidt, T., Jahns, P., Leister, D. **(2005)**. "Photosystem II core phosphorylation and photosynthetic acclimation require two different protein kinases." Nature 437(7062): 1179-1182.

In dieser Arbeit wird gezeigt, dass die plastidär lokalisierte Kinase STN8 in *Arabidopsis* für die Phosphorylierung von Untereinheiten des Photosystems II verantwortlich ist und dass diese Phosphorylierung nicht geschwindigkeitsbestimmend für Abbau und Neusynthese von D1 ist. Des Weiteren wurden in dieser Arbeit Arabidopsis *knock-out*-Linien für die Proteine von STN7, STN8 und die Doppelmutante *stn7/stn8* mittels des im Manuskript I entwickelten und optimierten Ausleseverfahrens untersucht. Im Gegensatz zum Wildtyp und der *stn8-knock-out*-Linie zeigten weder die *stn7-knock-out*-Linie noch die Doppelmutante *stn7/stn8* eine Veränderung von Chlorophyllfluoreszenzparametern und Pigmentzusammensetzung. Dadurch war ersichtlich, dass die STN7-Kinase nicht nur für die Kurzzeitantwort, sondern auch für die Langzeitantwort essentiell ist.

LETTERS

Photosystem II core phosphorylation and photosynthetic acclimation require two different protein kinases

Vera Bonardi[1,2]*, Paolo Pesaresi[2,3]*, Thomas Becker[1], Enrico Schleiff[1], Raik Wagner[4], Thomas Pfannschmidt[4], Peter Jahns[5] & Dario Leister[1,2]

Illumination changes elicit modifications of thylakoid proteins and reorganization of the photosynthetic machinery. This involves, in the short term, phosphorylation of photosystem II (PSII) and light-harvesting (LHCII) proteins. PSII phosphorylation is thought to be relevant for PSII turnover[1,2], whereas LHCII phosphorylation is associated with the relocation of LHCII and the redistribution of excitation energy (state transitions) between photosystems[3,4]. In the long term, imbalances in energy distribution between photosystems are counteracted by adjusting photosystem stoichiometry[5,6]. In the green alga *Chlamydomonas* and the plant *Arabidopsis*, state transitions require the orthologous protein kinases STT7 and STN7, respectively[7,8]. Here we show that in *Arabidopsis* a second protein kinase, STN8, is required for the quantitative phosphorylation of PSII core proteins. However, PSII activity under high-intensity light is affected only slightly in *stn8* mutants, and D1 turnover is indistinguishable from the wild type, implying that reversible protein phosphorylation is not essential for PSII repair. Acclimation to changes in light quality is defective in *stn7* but not in *stn8* mutants, indicating that short-term and long-term photosynthetic adaptations are coupled. Therefore the phosphorylation of LHCII, or of an unknown substrate of STN7, is also crucial for the control of photosynthetic gene expression.

STT7 and STN7 are orthologous protein kinases required for LHCII phosphorylation and for state transitions in *Chlamydomonas* and *Arabidopsis*, respectively[7,8]. In *Arabidopsis*, another STT7/STN7-like protein (STN8) exists that is not required for state transitions[8]. STN8 is located in the chloroplast, as shown by *in vivo* subcellular localization of its amino-terminal region fused to the dsRED protein and by the import of, and transit peptide removal from, STN8 translated *in vitro* (Fig. 1a, b). Chloroplast subfractionation after import revealed that the protein is associated, like STT7 and STN7, with thylakoids (Fig. 1c) (refs 7, 8).

Insertion mutants for *STN8* and *STN7* were obtained from the Salk collection[9], and for each gene two independent mutant alleles lacking the respective transcript were identified (Supplementary Fig. S1). The *stn7 stn8* double mutant was generated by crossing *stn7* and *stn8* single knockouts and screening the resulting F_2 generation for homozygous double mutants. All mutants were indistinguishable from the wild type with regard to the timing of seed germination and growth rate in the greenhouse (Supplementary Fig. S1). In *stn7* and *stn7 stn8* mutants, a slight decrease in the levels of neoxanthin, lutein and total chlorophyll was found (Supplementary Table S1). These subtle changes can be attributed to a minor decrease in LHCII content, not detectable by polyacrylamide-gel electrophoresis (PAGE) analysis (Supplementary Fig. S2).

Photosynthetic electron flow, measured on the basis of chlorophyll fluorescence, was not altered in the mutants (Supplementary Table S2). State transitions were suppressed in *stn7* and *stn7 stn8* plants (Supplementary Table S2) but were not affected in the *stn8* mutant, confirming previous results[8]. Reversible LHCII phosphorylation, which is associated with state transitions[7], was studied *in vivo* in dark-adapted plants incubated with [^{33}P]orthophosphate and then exposed to different light conditions (Fig. 2a). Under low light, wild-type and *stn8* plants showed a marked increase in LHCII phosphorylation, whereas subsequent exposure to high light decreased the amount of phospho-LHCII. In *stn7* and *stn7 stn8* plants, reversible phosphorylation of LHCII was not detectable, again in agreement with previous results[8]. Determination of LHCII phosphorylation with a phosphothreonine-specific antibody (Fig. 2b), or an *in vitro* assay in conditions under which the LHCII

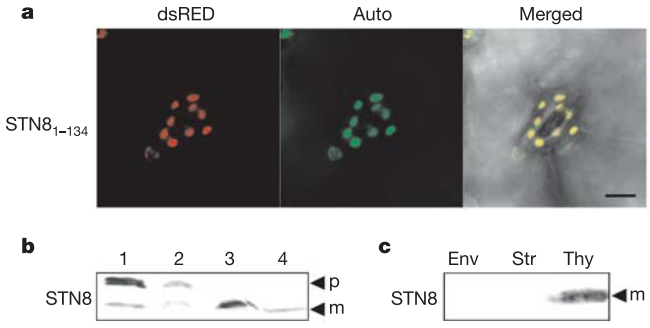

Figure 1 | Subcellular localization of STN8. a, The STN8$_{(1–134)}$–dsRED fusion was stably introduced into *stn8* plants. Guard cells were analysed by confocal laser scanning microscopy. Left, dsRED fluorescence identifying the fusion protein; middle, chloroplasts revealed by chlorophyll autofluorescence (shown in false colour); right, merged images. Scale bar, 50 μm. **b**, ^{35}S-labelled protein, translated *in vitro* (lane 1, 10% translation product) was incubated with isolated chloroplasts for 20 min at 4 °C (lane 2) or 25 °C (lanes 3 and 4), and chloroplasts were recovered by centrifugation through 40% Percoll. Chloroplasts were incubated with thermolysin (lane 4) and subjected to SDS–PAGE; proteins were detected by autoradiography. p, precursor; m, mature protein. **c**, Chloroplasts were fractionated after protein import. Env, envelope; Str, stroma; Thy, thylakoids.

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kinase should be maximally active (Fig. 2c), also showed that a loss of STN7 function, but not of STN8 function, suppresses the phosphorylation of LHCII. These results, together with the spectroscopic data, indicate that STN8 and STN7 do not act in series in phosphorylating LHCII.

Thylakoid protein accumulation was similar in mutant and wild-type plants (Supplementary Fig. S2). When phosphorylation of PSII core proteins was monitored with the phosphothreonine-specific antibody, *stn7* plants behaved like the wild type, showing high levels of phosphorylated PSII core proteins under all light regimes whereas LHCII phosphorylation was greatly decreased (Fig. 2d, e). In contrast, *stn8* plants showed a marked decrease in the total amount of PSII core phosphoproteins—particularly under high light—whereas LHCII phosphorylation was as in the wild type. This again argues that the main substrates of STN7 and STN8 are different: LHCII phosphorylation is mostly dependent on STN7, whereas phosphorylation of PSII core proteins depends almost exclusively on STN8. Strikingly, only in the *stn7 stn8* mutant were the phosphorylated forms of LHCII and the PSII core proteins completely absent under all light regimes tested, indicating that the two kinases must show some degree of overlap in their substrate specificities. However, the clear distinction between the phosphorylation phenotypes of the two mutants implies that STN7 and STN8 act in parallel and could be directly responsible for phosphorylating the LHCII and PSII core proteins, respectively. If, however, the proposal that LHCII is phosphorylated by thylakoid-associated kinase (TAK) proteins[10] is correct, then STN7 might act upstream of TAKs.

Reversible phosphorylation of the D1 protein is thought to have a key function in the regulation of its turnover during the photoinhibition of PSII (ref. 11). Exposure to light induces phosphorylation of, and causes damage to, PSII reaction centres, and the phosphorylated form of damaged D1 is resistant to proteolysis[12]. However, relocation of damaged PSII centres from grana to stroma lamellae permits the dephosphorylation and proteolysis of D1, and co-translational incorporation of newly synthesized D1 (ref. 1). Consequently, lack of the D1 protease impairs the PSII repair cycle[13]. To test whether suppression of PSII core phosphorylation also impairs PSII repair by changing the rate of D1 turnover, we investigated the inactivation of PSII under high light, the recovery of PSII activity and the degradation of D1. Illumination of leaves at high light intensity led to a slightly stronger inactivation of PSII in *stn8* and *stn7 stn8* plants than in *stn7* and wild-type plants; the subsequent recovery of PSII activity under low light was also somewhat slower in *stn8* and *stn7 stn8* than in wild-type and *stn7* plants (Fig. 3a). However, the increased photosensitivity of PSII in *stn8* and *stn7 stn8* plants was not reflected in changes in the rate of light-induced D1 degradation in the presence of lincomycin, an inhibitor of plastid protein synthesis (Supplementary Fig. S3). Moreover, also during photoinhibition and subsequent recovery, no PSII core phosphorylation was detected in *stn7 stn8* plants (Supplementary Fig. S4), excluding the action of another PSII core kinase under these conditions. Pulse–chase experiments under high light intensities

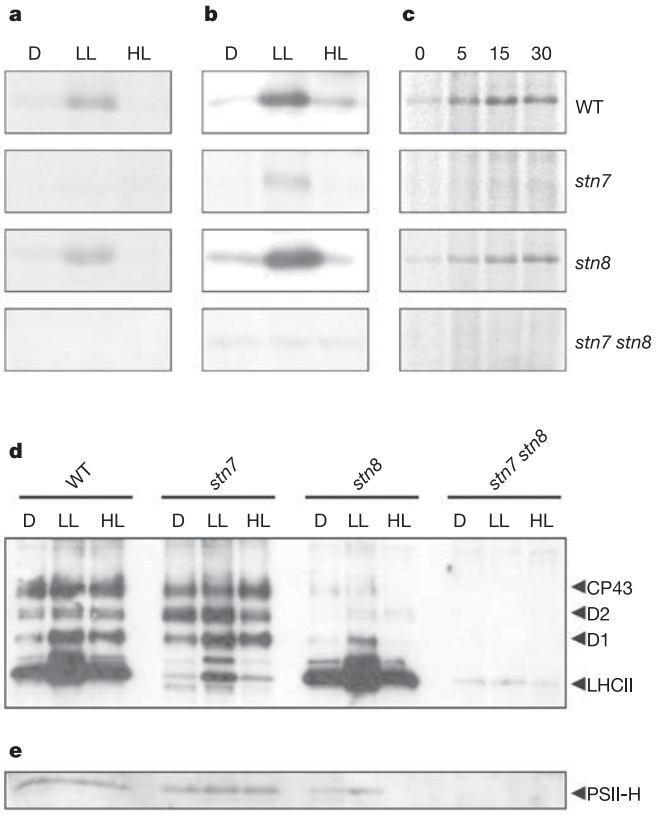

Figure 2 | Phosphorylation of thylakoid proteins. **a**, *In vivo* LHCII phosphorylation. Leaves were incubated with [^{33}P]orthophosphate, and thylakoid proteins from plants kept in the dark (D), subsequently exposed to low light (LL), and then to high light (HL), were fractionated by SDS–PAGE. WT, wild type. **b**, LHCII phosphorylation detected by immunoblot analysis with a phosphothreonine-specific antibody. Leaves were treated and proteins fractionated as in **a**. **c**, *In vitro* LHCII phosphorylation. Thylakoids from dark-adapted leaves were incubated with [γ-^{33}P]ATP under reducing conditions for 0, 5, 15 and 30 min in the dark. Proteins were fractionated as in **a**. **d**, **e**, Phosphorylation of LHCII and PSII core proteins detected by immunoblot analysis. Leaves were treated and thylakoid proteins fractionated as in **b**.

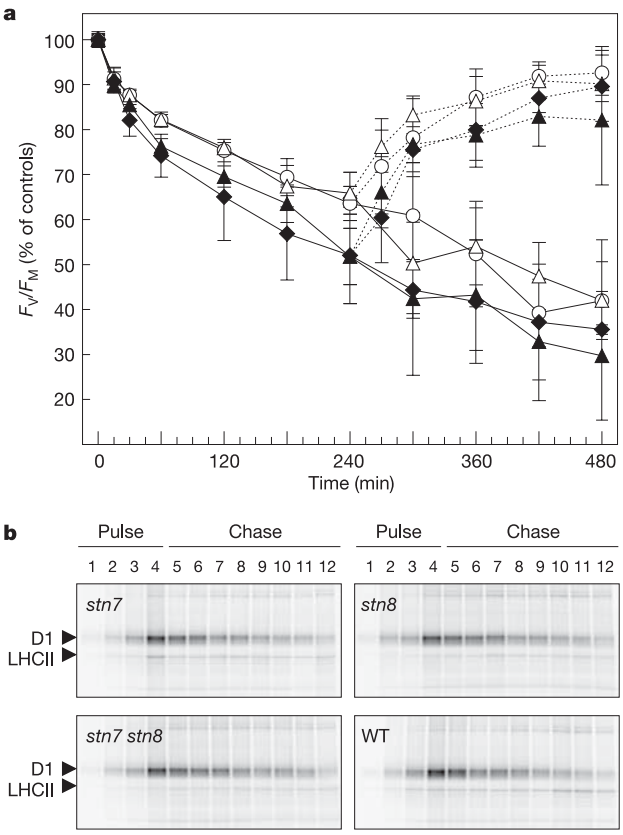

Figure 3 | PSII activity and D1 turnover under high-intensity light. **a**, Time course of PSII inactivation (solid lines) induced by high light (2,000 μmol photons m^{-2} s^{-1}) and recovery under low light (20 μmol photons m^{-2} s^{-1}; dashed lines). Error bars indicate s.d. Open triangles, *stn7*; filled triangles, *stn8*; diamonds, *stn7 stn8*; circles, wild type. **b**, Autoradiogram of thylakoid membrane proteins resolved by SDS–PAGE after pulse-labelling with [^{35}S]methionine for 0, 15, 30 and 60 min (lanes 1–4) and subsequent chase in unlabelled medium for 60, 120, 180, 240, 300, 360, 420 and 480 min (lanes 5–12), both under high light (2,000 μmol photons m^{-2} s^{-1}). WT, wild type.

revealed that rates of D1 synthesis and degradation were similar in all genotypes (Fig. 3b; Supplementary Fig. S5). In addition, wild-type, *stn8* and *stn7 stn8* plants grown under high light intensities behaved very similarly in growth rate and leaf pigment composition (data not shown). Taken together, the data indicate that STN8-mediated phosphorylation of D1 is not crucial for D1 turnover and PSII repair.

Changes in light conditions are thought to result, in the long term, in the adjustment of photosystem stoichiometry, which requires a signalling network that coordinates photosynthetic gene expression in plastids and nucleus[5,6,14]. Recent studies have proposed a functional relationship between LHCII phosphorylation (and state transitions) on the one hand, and the long-term response to altered light conditions on the other[15,16]. The slower growth of *stn7* mutants under fluctuating light has been attributed to impaired state-transition-based adaptation[8]. Here we tested whether defects in the function of STN7 or STN8 affect the changes in antenna structure and photosystem stoichiometry that are associated with the long-term response to changes in the quality of incident light[17,18]. Plants were grown and acclimated to light sources that favoured either PSI or PSII, and the long-term response was followed by monitoring the chlorophyll fluorescence parameter F_S/F_M and the ratio of chlorophyll *a* to chlorophyll *b* (Chl *a*/*b*). In the wild type, F_S/F_M values were relatively high after acclimation to PSI light, and low values were measured after acclimation to PSII light, whereas the Chl *a*/*b* ratio behaved in the opposite sense, being high after acclimation to PSII light and low in PSI light[17,18] (Fig. 4a, b). The *stn8* mutant behaved like the wild type (Fig. 4a, b), indicating that its long-term response was normal. In the *stn7* and *stn7 stn8* mutants, however, F_S/F_M and Chl *a*/*b* values were typical of plants acclimated to PSI light under all light regimes tested, indicating that the mutants have lost their capacity for the long-term response and implying that STN7 has a function in coordinating the long-term and short-term responses to changes in light conditions.

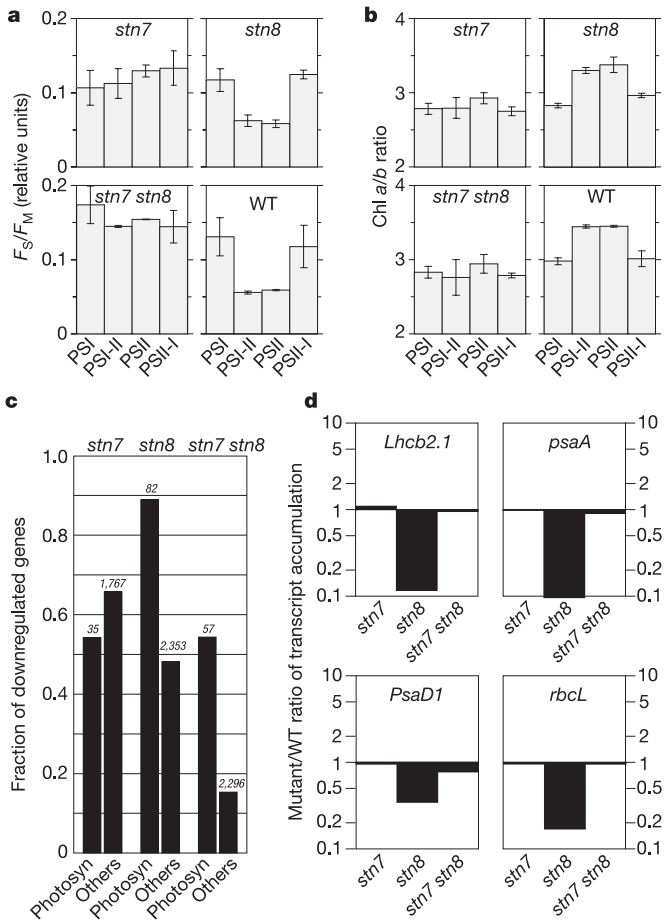

Figure 4 | Photosynthetic acclimation and mRNA expression. a, **b**, Plants were acclimated to PSI or PSII light and F_S/F_M values (**a**) and Chl *a*/*b* ratios (**b**) were determined. Error bars indicate s.d. WT, wild type. **c**, Nuclear transcript accumulation in greenhouse-grown plants. Numbers above bars indicate the total numbers of genes that are significantly differentially expressed in mutants with respect to wild-type plants. Bar lengths indicate the fraction of differentially regulated genes that were downregulated. Photosyn, genes for photosynthesis; others, other genes (for non-photosynthetic proteins). **d**, Mutant versus wild-type ratios of transcript levels (logarithmic scale) of selected nuclear and plastid photosynthetic genes.

Lack of STN7 might impair the long-term response by interfering with a signalling pathway that links changes in photosynthetic efficiency during the adjustment of photosystem stoichiometry to the level of gene expression in plastids[19] and the nucleus[17,18]. To test this we analysed the transcription of photosynthetic genes. For nuclear genes encoding chloroplast proteins two modes of transcriptional regulation are known: a master switch that acts on most genes[20] and an additional mechanism specific for photosynthetic genes[21]. Lack of STN7 in greenhouse-grown *stn7* and *stn7 stn8* plants results in the differential expression of only relatively few photosynthetic genes, in contrast to *stn8* plants in which a large set of photosynthetic genes is markedly downregulated (Fig. 4c; Supplementary Table S3); exemplary expression profiles of two nuclear and two plastid genes are provided in Fig. 4d. The impairment of the transcriptional regulation of certain *stn8*-responsive genes in the absence of STN7, together with the results of the long-term response experiments, therefore argues in favour of a function for STN7 in the regulation of nuclear and plastid gene expression. We can only speculate how STN7 triggers changes in photosynthetic gene expression but, in principle, three hypotheses are available: first, the phosphorylation state of LHCII directly provides information for signalling; second, an unknown protein is phosphorylated by STN7 and participates in signalling; and third, state transitions and the associated conformational changes of thylakoids[3,22] stimulate signalling.

In green algae, the pool of mobile LHCII is large[23], and state transitions are important for cyclic electron flow[3]. Because the mobile LHCII pool in vascular plants is relatively small[24], it is tempting to speculate that triggering of the long-term response, rather than the short-term response in terms of state transitions, represents the major function of STN7 in flowering plants. In this respect, the slower growth of *stn7* plants under fluctuating light[8] might be due to disturbance of transcriptional regulation rather than being a physiological consequence of defects in state transitions. Future analyses must clarify how the short-term and long-term responses are coupled, and whether STN7 and STN8 are necessary and sufficient for the phosphorylation of thylakoid proteins.

METHODS

Plant lines and propagation. Mutant lines from the Salk collection[9] were identified by searching the SiGNAL database (http://signal.salk.edu/tabout.html). Methods for plant propagation have been described elsewhere[25–27].

Chlorophyll fluorescence and pigment analysis. Photosynthetic electron transport, state transitions and leaf pigment composition were measured as described[25–27] (see Supplementary Information for details).

Analysis of LHCII phosphorylation *in vivo* and *in vitro*. To determine the degree of LHCII phosphorylation *in vivo*, dark-adapted leaves from 4-week-old plants were incubated in the presence of [^{33}P]orthophosphate for 1 h and subsequently exposed for 2 h to levels of illumination favouring phosphorylation (low light, 80 μmol photons m^{-2} s^{-1}) or dephosphorylation (high light, 800 μmol photons m^{-2} s^{-1}). Identical amounts of thylakoid proteins (equivalent to 200 mg of fresh leaf) were prepared in the presence of 10 mM NaF[25] and fractionated by SDS–PAGE (14% polyacrylamide); labelled proteins were detected by phosphorimaging (Typhoon; Amersham Biosciences). For the assay *in vitro*, thylakoids isolated from dark-adapted leaves (30 mg of fresh leaf) were incubated with [γ-^{33}P]ATP under reducing conditions in the dark[25]. Separation and detection of thylakoid proteins were performed as described for the *in vivo* assay.

Immunoblot analysis. Leaves from 4-week-old plants were harvested after overnight dark adaptation or light exposure as described above. Thylakoids

were prepared in the presence of 10 mM NaF, fractionated on an SDS–polyacrylamide-gradient gel (8–25% polyacrylamide) and transferred to poly(vinylidene difluoride) membranes[25]. Filters were then probed with antibodies specific for phosphothreonine (Biolabs) and signals were detected by enhanced chemiluminescence (Amersham Biosciences).

Pulse–chase measurement of D1 turnover. The pulse–chase procedure for the analysis of D1 turnover in pea[28] was modified for *Arabidopsis*. For radioactive labelling of thylakoid proteins, leaf discs of 3-week-old *Arabidopsis* plants were pressed extremely gently against coarse sand paper and then vacuum-infiltrated in a syringe containing 1 mCi of L-[^{35}S]methionine in 10 ml of 1 mM KH_2PO_4 pH 6.3, 0.1% Tween 20. Directly after infiltration, three leaf discs were frozen in nitrogen (t_0). Remaining leaves were transferred to high light (2,000 μmol photons $m^{-2}\,s^{-1}$) and for each time point ($t_{pulse} = 15$, 30 and 60 min) three leaf discs were collected. Immediately after the pulse period, the remaining leaf discs were washed, incubated with 10 mM unlabelled L-methionine in the same buffer as before and further exposed to high light for up to 8 h ($t_{chase} = 60$, 120, 180, 240, 300, 360, 420 and 480 min). The three leaf discs for each time point were combined and thylakoid proteins were prepared, separated and detected as described for the LHCII phosphorylation analysis.

***In vitro* import and intracellular localization of dsRED fusions in chloroplasts.** For *in vitro* import assays, ^{35}S-labelled proteins were synthesized and used for import experiments and were detected after subfractionation of chloroplasts[29,30]. For intracellular localization of the dsRED fusion, a complementary DNA fragment coding for the first 134 amino-acid residues of STN8 was fused 5′ to *dsRED* and inserted into the vector pLEELA (Invitrogen), placing it under the transcriptional control of the cauliflower mosaic virus 35S promoter. Seeds were collected from transformed *stn8* plants[26] and independent transgenic plants were selected. Confocal images were collected by laser scanning microscopy (TCS SP2; Leica). Fluorescence was excited with a 461 nm HeNe laser and images were collected in the ranges 565–620 nm (dsRED fluorescence) and 670–750 nm (chlorophyll autofluorescence).

Nucleic acid analysis. *Arabidopsis* DNA was isolated[26] and T-DNA insertion junction sites were recovered by polymerase chain reaction (PCR) with the use of combinations of insertion-specific and gene-specific primers, and then sequenced. To determine levels of gene expression, extraction of total leaf RNA, first-strand cDNA synthesis and reverse-transcriptase-mediated PCR (RT–PCR) were performed[26], using primers specific for *STN7* or *STN8*, as well as *ACTIN1*-specific oligonucleotides as a control.

mRNA expression profiling. Greenhouse-grown mutant and wild-type plants were analysed. The generation and use of a 3292-gene sequence tag (GST) nylon array enriched for nuclear genes for chloroplast proteins, data analysis and statistical evaluation have all been described previously[20,21,26] (see Supplementary Information for details).

Measurement of acclimation to changes in light quality. Light conditions favouring either PSI or PSII (PSI or PSII light, respectively), as well as growth and acclimation conditions for *Arabidopsis*, have been described previously[18]. Plants were initially grown for 10 days under white light followed by a 6-day acclimation period. Seedlings were acclimated either to PSI or PSII light for 6 days or to PSI light for 2 days followed by 4 days under PSII light or vice versa. Measurement of Chl *a*/*b* ratios and determination of the chlorophyll fluorescence parameters F_S (steady-state fluorescence) and F_M (maximal fluorescence), and of F_S/F_M ratios, were performed as described in Supplementary Information. All values under the four conditions were calculated as the means of 50 individuals in at least three independent experiments, and the significance of differences between samples was tested with Student's *t*-test.

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Supplementary Information is linked to the online version of the paper at www.nature.com/nature.

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Author Information Complete data sets are deposited at GEO (http://www.ncbi.nlm.nih.gov/geo/) under accession numbers GSE2620–GSE2622. Reprints and permissions information is available at npg.nature.com/reprintsandpermissions. The authors declare no competing financial interests. Correspondence and requests for materials should be addressed to D.L. (leister@lrz.uni-muenchen.de).

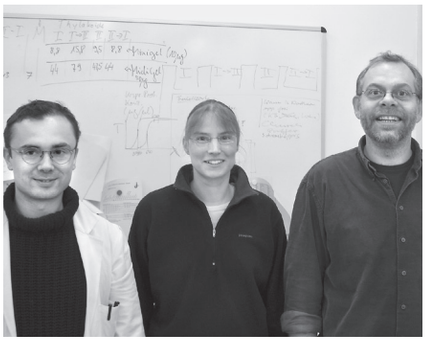
V

Wagner, R., Bräutigam, K., Pfannschmidt, T. **(2006)**. "Die Photosynthese – Ein Umweltsensor, der Gene reguliert." Bioforum 3: 48-50.

In diesem kurzen deutschen Übersichtsartikel wurden die aktuellen Entwicklungen für ein breites Publikum zusammengefasst, nämlich dass der Prozess der Photosynthese gleichermaßen chemische Energie als auch Informationen an die Pflanze liefert. Diese Informationen scheinen von der Pflanze interpretierbar zu sein, da diese dann sinnvoll auf Umweltveränderungen reagiert, in dem sie ihre Proteinausstattung modifiziert und erneuert.

Die Photosynthese

Ein Umweltsensor, der Gene reguliert



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Die Photosynthese wird sehr stark durch die Umwelt beeinflusst. Besonders sensibel reagiert der Elektronentransport der Lichtreaktion, wobei Umweltveränderungen sich in Verschiebungen der Reduktions- bzw. Oxidationszustände von den Komponenten der Transportkette widerspiegeln. Neueste Daten zeigen, dass über diese Redox-Änderungen gezielt die Expression von Genen für Anpassungsreaktionen ge steuert wird. Die Photosynthese fungiert somit als Sensor für die pflanzliche Um welt, der auf molekularer Ebene die eigene Akklimation steuert.

Vielfältige Umweltfaktoren beeinflussen die Photosynthese

Photoautotrophe Organismen wandeln die Sonnenenergie in Kohlenhydrate um und machen sie so für sich selbst aber auch für die nicht-photosynthetischen Organismen chemisch zugänglich. Gewissermaßen nebenbei liefert dieser Vorgang auch den Sauerstoff, den wir atmen. Die molekularen Vorgänge bei dieser Umwandlung wurden in den letzten Jahrzehnten ausführlich erforscht und sind in den wesentlichen Zügen verstanden. Die aktuelle Forschung beschäftigt sich daher zunehmend mit dem Bereich der Regulation. Die dabei gestellten Fragen zielen vorwiegend darauf ab zu verstehen, wie photosynthetische Organismen selbst unter extremsten Bedingungen noch Photosynthese betreiben können und wie sie diesen komplexen Prozess an die sich ständig verändernde Umwelt anpassen. Besonders stark wirken sich Veränderungen in der Belichtung einer Pflanze aus, da diese direkt die Lichtreaktion der Photosynthese betreffen. Dabei können Stärke, spektrale Zusammensetzung und Dauer der Belichtung im Sekunden-, Minuten-, Stunden- bis hin zum saisonalen Bereich schwanken. Gleichzeitig ist die photosynthetische Lichtreaktion mit der nachfolgenden enzymabhängigen Dunkelreaktion eng gekoppelt und daher auch für Temperaturunterschiede sowie für Veränderungen in der Kohlendioxid-, Nährstoff- und Wasserverfügbarkeit empfänglich. Damit ist die Photosynthese ein Prozess, der durch multifaktorielle Veränderungen beeinflusst werden kann.

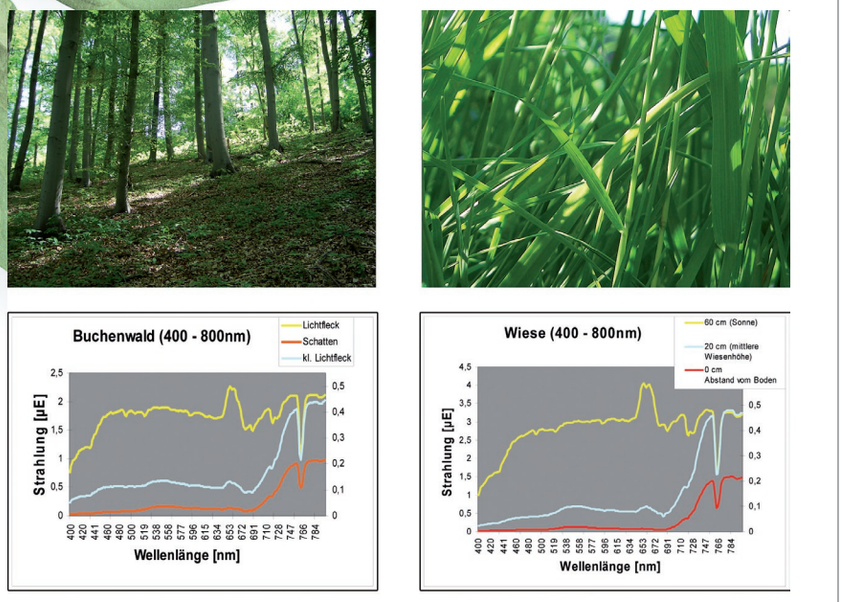

Abb. 1: Dichte Pflanzenbestände und ihre typischen Lichtbedingungen

Der Elektronentransport reagiert sensibel auf ungleiche Anregung der Photosysteme

In höheren Pflanzen findet die Photosynthese in einem speziellen Zellkompartiment, den Chloroplasten, statt. Diese enthalten dassog.Thylakoidmembransystem, in welches der Photosyntheseapparat eingelagert ist. Dieser besteht aus den integralen Multiproteinkomplexen Photosystem II (PSII), dem Cytochrom b_6f (Cytb_6f)-Komplex und dem Photosystem I (PSI). Verbunden sind diese Komplexe über die mobilen Elektronen-Carrier Plastochinon (PQ) und Plastocyanin (PC). Die Lichtenergie wird über spezielle Antennenkomplexe, die die Chlorophylle enthalten, gesammelt und in die Reaktionszentren geleitet, wobei PSII und PSI leicht unterschiedliche Absorptionsmaxima (680 bzw. 700 nm) auf weisen. Am PSII werden in einer ersten Lichtreaktion H_2O-Moleküle in Protonen, Elektronen und Sauerstoff zerlegt. Die Elektronen werden dann entlang eines Redoxpotential-Gradienten auf PSI übertragen, wo sie mit Hilfe einer zweiten Lichtreaktion auf den Endakzeptor $NADP^+$ übertragen werden (linearer Elektronentransport). Das so entstandene Reduktionsäquivalent wird dann in der anschließenden Dunkelreaktion verwendet. Elektrochemisch gesehen wirken dabei die zwei Photosysteme in Serie. Eine ungleichmäßige Anregung der Photosysteme kann daher zu einem Ungleichgewicht in der Verteilung der Anregungsenergie zwischen den beiden Systemen führen, was massiv den linearen Elektronentransport beeinflusst und zu einer Herabsetzung der Photosyntheseeffizienz führt. In dich-

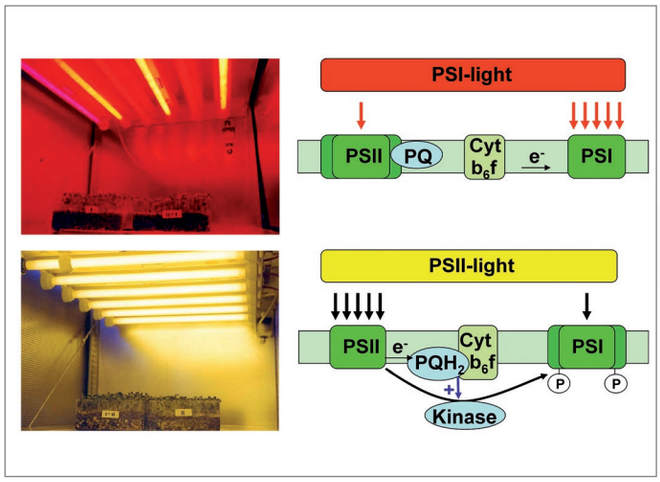

Abb. 2: Künstliche PSII- und PSI-Lichtquellen und ihr Einfluss auf den linearen Elektronentransport und die state transition

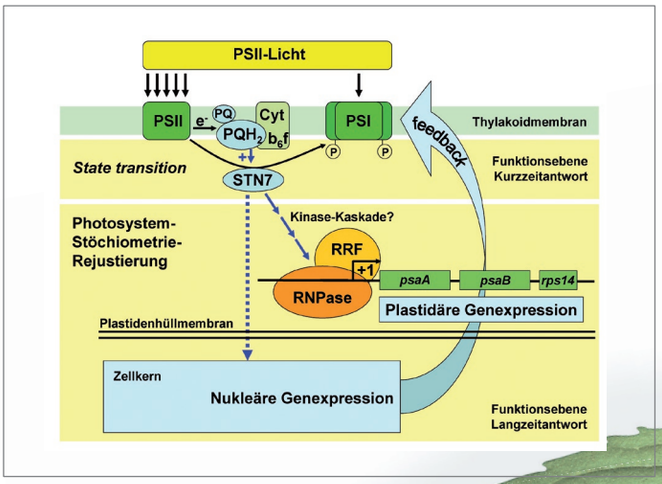

Abb. 3: Arbeitsmodell zur molekularen Regulation von state transition und LTR

ten Pflanzenbeständen treten starke Lichtqualitätsgradienten auf, die genau dies bewirken. Man beobachtet dabei eine starke Anreicherung roter Wellenlängen, die aufgrund seines höheren Absorptionsmaximums zu einer stärkeren Anregung von PSI gegenüber PSII führen (Abb. 1). Dies resultiert in einem vornehmlich oxidierten Zustand der Elektronentransportkette. Um dem entgegen zu wirken, haben Pflanzen Mechanismen zur Energieumverteilung entwickelt. Man unterscheidet dabei zwischen Kurzzeitantworten, die im Minutenbereich aktiv sind und Langzeitantworten, die mehrere Stunden bis hin zu Tagen brauchen. Wir ahmen solche natürlichen Lichtgradienten im Labor mit Hilfe spezieller Lichtquellen (PSI- bzw. PSII-Licht, Abb. 2) nach. Indem wir Pflanzen unter diesen Lichtquellen anziehen, können wir deren Kurz- und Langzeitantworten auf Lichtqualitätsgradienten studieren.

Eine gut untersuchte Kurzzeitantwort ist die sog. state transition [1]. In ihr wird ein Teil der Licht sammelnden Antennenkomplexe des PSII, der sog. LHCII (light harvesting complex), zwischen den beiden Photosystemen verschoben. Hierdurch wird der relative Antennenquerschnitt der beiden Photosysteme aktiv verändert und damit ein Teil der eingefangenen Lichtenergie umdirigiert. Diese Verschiebung wird über eine Phosphorylierung der Proteine des LHCII reguliert, die durch eine spezifische LHCII-Kinase erfolgt. Diese wiederum wird durch den Redox-Zustand des PQ-Pools (transportiert die Elektronen von PSII zum Cytb_6f-

Komplex) kontrolliert (Abb. 2). Bei zunehmender Reduktion des Pools (z.B. wenn PSI zuwenig angeregt wird) wird sie aktiviert und phosphoryliert den mobilen Teil des LHCII. Dieser bewegt sich daraufhin lateral zum PSI und leitet so mehr Anregungsenergie in dieses Photosystem. Bei zunehmender Oxidation des Pools dagegen geschieht das Gegenteil. Diese redox-sensitive Kinase konnte nach fast 30 Jahren Suche kürzlich eindeutig identifiziert werden und wurde STN7 genannt [2].

In einer langfristigen Akklimationsreaktion (long term response, LTR) wird die relative Anzahl von PSI zu PSII variiert. Diese Verschiebung der Photosystem-Stöchiometrie hat den gleichen aber länger anhaltenden Effekt auf die Umdirigierung der Anregungsenergie wie die oben beschriebene state transition. Während letztere eine physiologische Reaktion auf post-translationaler Ebene darstellt, erfordert die dynamische Veränderung der Photosysteme eine tiefer gehende Regulation, die bis auf die Ebene der Genexpression reicht. Forschungen der letzten Dekade zeigen, dass dieser Prozess in allen bisher unter suchten Organismen von Cyanobakterien über Grünalgen bis hin zu höheren Pflanzen zu finden ist [3]. Wie in der state transition spielt der Redox-Zustand des PQ-Pools eine entscheidende Rolle bei der Regulation [4]. Reduktion zeigt eine limitierende Funktion des PSI, Oxidation eine limitierende Funktion des PSII an. Die Stöchiometrie wird deswegen zugunsten des jeweilig limitierenden Photosystems verschoben. Dabei wird spezifisch die Expression der Gene für die Kernproteine der Photosystem-Komplexe reguliert, die offensichtlich eine Art pace maker Funktion bei der Synthese und Assemblierung des Komplexes erfüllen. Diese Gene sind in höheren Pflanzen und Grünalgen universell in den Chloroplasten kodiert und ihre Expression kann so von jedem Chloroplasten individuell gesteuert werden. Der größte Teil der Proteinausstattung der Chloroplasten, und dies betrifft auch den Photosyntheseapparat, ist aber im Zellkern kodiert. Mehrere Labors konnten zeigen, dass photosynthetische Redox-Signale über noch unbekannte Signalwege aus dem Chloroplasten heraus gelangen und aktiv auch Gene im Zellkern steuern [5]. Sie stellen damit eine ganz neue Klasse von Signalen dar, die bei der retrograden Kommunikation, also der Signalleitung von Zellorganellen zum Zellkern, eine wichtige Rolle spielen.

Einen Durchbruch erzielten wir jüngst im Verständnis der funktionellen Relation von state transition und LTR. Zusammen mit unserem Kooperationspartner Prof. Dr. Dario Leister von der LMU München konnten wir zeigen, dass Pflanzen, denen die LHCII-Kinase STN7 fehlt, nicht nur keine state transition mehr besitzen, sondern auch die LTR nicht mehr zeigen [6]. Dies zeigt, dass Kurzzeit- und Langzeitantwort gekoppelte Prozesse sind, wobei die state transition wahrscheinlich das Eingangssignal für die LTR triggert (Abb. 3).

Bei photosynthetischen Akklimationsreaktionen handelt es sich also um eine funktionelle feedback-Schleife, über die die Photosynthese die Expression ihrer eigenen Gene, aber auch die gekoppelter Prozesse, reguliert und an ihren jeweiligen Funktionszustand anpasst. Die Photosynthese stellt also nicht einfach nur einen passiven, Energie fixierenden Prozess dar, sondern sie wirkt gleichzeitig auch als Umweltsensor, der, wenn nötig, regulierend in die Expression von Genen eingreift. Dabei stellen die hier beschriebenen Mechanismen nur einen kleinen Ausschnitt aus einer Vielzahl von Redox-regulierten Antworten der Photosynthese dar. Einige davon (einschließlich unseres Projektes) werden in einer DFG-Forschergruppe (FOR 387) gefördert und sind auf der homepage der Forschergruppe detailliert beschrieben (www.uni-bielefeld.de/biologie/fg387/index.html).

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VI

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In dieser Veröffentlichung wird erstmals geschätzt, dass zwischen 50 und 100 eukaryotische Transkriptionsfaktoren in Plastiden importiert werden. Weiterhin stellen wir ein Modell vor, welches die Möglichkeit skizziert, wie die Substitution ursprünglich in Plastiden vorhandener Signalwege und Transkriptionsfaktoren erfolgt sein könnte.

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Eukaryotic transcription factors in plastids — Bioinformatic assessment and implications for the evolution of gene expression machineries in plants

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Abstract

The expression of genes in higher plant chloroplasts includes a complex transcriptional regulation which can be explained only in part with the action of the actually known components of the transcriptional machinery. This suggests the existence of still unknown important regulatory factors which influence chloroplast transcription. In order to test if such factors could exist we performed *in silico* analyses of *Arabidopsis* genes encoding putative transcription factors looking for putative N-terminal chloroplast transit peptides in the amino acid sequences. Our results suggest that 48 (and maybe up to 100) transcription factors of eukaryotic origin are likely to be imported into plastids. None of them has been described yet. This set of transcription factors highly expands the actually known regulation capacity of the chloroplast transcription machinery and provides a possible explanation for the complex initiation patterns of chloroplast transcripts. As consequence of a massive import of eukaryotic transcription factors a comprehensive reconstruction of the ancient prokaryotic gene expression machinery must be assumed resulting in a novel compatible combination of eukaryotic and prokaryotic protein components. In turn, the opposite process has been induced in the nucleus by the integration of prokaryotic components of the plastid ancestor *via* its loss of genes during endosymbiosis. Thus, a mutual exchange of regulatory factors, i.e. transcription factors occurred which resulted in the unique signalling network of todays plants. An evolutionary model of how this could have emerged during endosymbiosis in a timely coordinated manner is proposed.

Keywords: DNA-binding proteins; Chloroplast transcription factors; Evolution; Genetic system; *Arabidopsis thaliana*

1. Introduction

Chloroplasts of higher plants contain their own genome which encodes a relatively stable subset of 100–120 genes. These encode mainly components of the photosynthetic apparatus and the plastid gene expression machinery (Sugiura, 1995). Transcription of plastid genes is performed by two different RNA polymerases, a nuclear encoded polymerase (NEP) and a plastid encoded polymerase (PEP) (Liere and Maliga, 2001). The NEP is a single subunit enzyme of the T7 phage type, while the PEP is a multisubunit enzyme of bacterial origin with a subunit stoichiometry of α_2, β, β′, β″ forming the so-called core enzyme (Igloi and Kössel, 1992; Hess and Börner, 1999). The NEP enzyme exclusively transcribes the *rpo* genes (encoding the PEP subunits α, β, β′, β″), the *clpP* and the *accD* gene. It recognizes a specific YRTA motif which can be found also in front of several photosynthesis gene operons (Liere and Maliga, 1999). Early models assumed that the NEP is mainly active in proplastids during early seed development transcribing house keeping genes while the PEP is the predominant enzyme activity in chloroplasts transcribing photosynthesis and most other genes. However, present data favour a parallel role for both RNA polymerases in all plastid types (Liere and Maliga, 2001).

PEP promoter sequences contain bacteria-like promoter *cis*-elements like the −10 and −35 region. Like the bacterial ancestor the core enzyme of PEP requires the interaction with a σ-factor for transcription initiation at these sites. This interaction generates a so-called holoenzyme which is capable of specific

Abbreviations: cTP, chloroplast transit peptide; NLS, nuclear localisation sequence; TF, transcription factor.

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promoter recognition (Link, 1996; Allison, 2000; Suzuki et al., 2004). In *Arabidopsis* six different σ-factors have been identified so far which are active in a development- or tissue-specific manner (Kanamaru and Tanaka, 2004). Knock-out or antisense plants defective in σ-factors exhibit only weak or transient phenotypes such as pale cotyledons while the adult plants appeared normal (Privat et al., 2003; Ishizaki et al., 2005). This indicates that lacking σ-factor activities can be easily compensated by either the residual σ-factors or by other components. The only exception appears to be SIG2. A knock-out of this σ-factor strongly affects the light-dependent chloroplast development and can be restored only by complementation with the wild-type gene (Kanamaru et al., 2001). SIG2 is responsible for proper expression of several plastid tRNA genes including *trnE* encoding the glutamyl-tRNA, a precursor molecule of chlorophyll synthesis. Recently it was reported that this molecules also binds the NEP enzyme *in vitro* suggesting a role in the down-regulation of NEP activity in mature chloroplasts (Hanaoka et al., 2005). In summary this points to an important role of σ-factors in the basic transcription machinery, however, this role appears to be limited mainly on early stages of plastid development.

Characterisation of promoter structures of many chloroplast operons in different plants detected multiple transcription initiation sites, e.g. five sites in front of the *psbD-psbC* operon of barley including one blue-light responsive promoter (BLRP) (Berends-Sexton et al., 1990), four promoters in front of the *atpB* operon of tobacco (Hajdukiewicz et al., 1997), three promoters in front of the 16 S rRNA gene (Baeza et al., 1991; Pfannschmidt and Link, 1997) or two promoters within the *psbK-psbI-trnG* cluster of tobacco (Meng et al., 1991). This multiplicity of initiation sites can be explained only in part by differential action of the NEP and PEP enzymes since many of those start sites have no respective upstream *cis*-elements. This suggests the involvement of additional regulatory protein factors conferring promoter specificity in chloroplast transcription. As a matter of fact a handful of additional transcription or DNA-binding factors have been described so far to be active in chloroplast promoter recognition such as CDF1 in tobacco, CDF2 in spinach, AGF in barley, two proteins of 36 and 31 kDa protein in rice, PGTF in barley, PTF1 in *Arabidopsis*, RLBP in tobacco and two Sig1 interacting proteins as well as two whirly proteins in *Arabidopsis* (Lam et al., 1988; Baeza et al., 1991; Kim and Mullet, 1995; Cheng et al., 1996; To et al., 1996a,b; Kim et al., 1999; Baba et al., 2001; Kim et al., 2002; Morikawa et al., 2002; Krause et al., 2005). In most cases also the specific binding site could be identified which often differed in their sequence from the typical NEP or PEP promoter elements described above (see Discussion). All these DNA-binding factors were identified by biochemical approaches analysing their interaction with the respective promoter region. The identity of these proteins, however, remained elusive so far. In addition to these factors, a number of nucleoid-binding proteins such as MFP1, DND41, PEND, DPC68, SiR, RPL4 and ET1 (Sato et al., 1993; Nakano et al., 1997; Trifa et al., 1998; Sato et al., 2001; Chi-Ham et al., 2002; Jeong et al., 2003; da Costa e Silva et al., 2004) were identified. Most of them seem to be involved in the structural integrity and membrane association of nucleoids.

PEP in its predicted bacterial subunit composition of α_2, β, β′, β″ could be purified so far only from young greening chloroplasts or etioplasts (Hu and Bogorad, 1990; Pfannschmidt and Link, 1994) while it exhibits a much more complex structure in mature chloroplasts (Lerbs et al., 1985; Rajasekhar et al., 1991; Lakhani et al., 1992; Pfannschmidt and Link, 1994; Boyer and Hallick, 1998; Suzuki et al., 2004). Recent mass spectrometric analyses of purified chloroplast PEP complexes identified all *rpo* gene products as well as a number of other subunits with additional functions such as RNA binding proteins, superoxide dismutase, kinase activities and several more (Pfannschmidt et al., 2000; Ogrzewalla et al., 2002; Loschelder et al., 2004; Suzuki et al., 2004; Pfalz et al., 2006). These additional factors suggest that in chloroplasts the former bacterial RNA polymerase acquired several new, mostly eukaryotic factors to meet the specific demands in mature chloroplasts. This might have ensured proper transcription during evolutionary adaptation of the cyanobacterial ancestor toward todays chloroplast. We therefore assumed that chloroplast RNA polymerase(s) might require additional, possibly eukaryotic transcription factors (TFs) for the regulation of transcription. Acquisition of eukaryotic factors by the genetic machinery of plastids has been proposed earlier, however, without specific emphasis on 'true' TFs (Sato, 2001). Recent development of new databases allowed us to investigate specifically this question by investigating *Arabidopsis* genes encoding putative or known TFs for the presence of chloroplast transit peptides. Our search suggests the existence of many not yet described eukaryotic TFs in chloroplasts. The results were discussed with respect to evolutionary implications for the development of plant gene expression machineries in plastids and nucleus.

2. Materials and methods

2.1. Data sources

A list with gene names of 6084 putative transcription factors encoded by the *Arabidopsis* genome was obtained from the Proteome database of Transcription Factors TrSDB (http://bioinf.uab.es/cgi-bin/trsdb/trsdb.pl; Aguilar et al., 2002). This list contains genes which exhibit similarities to known transcription factors as predicted by TranScout (http://luz.uab.es/TranScout) and which have homologues within other TrSDB proteomes. The amino acid sequences of these proteins were obtained as FASTA files from the TRCDSEMBL database using BioRS (http://biors.gsf.de:8111/searchtool/searchtool.cgi?start_guest_session=1) from MIPS (http://mips.gsf.de/). As second data source, 1826 protein sequences representing *Arabidopsis* transcription factors were obtained as FASTA files from the Database of Arabidopsis Transcription Factors DATF (http://datf.cbi.pku.edu.cn/index.php; Guo et al., 2005).

2.2. Bioinformatic analyses

All sequences were analysed for putative chloroplast directing N-terminal transit peptides with TargetP1.1 (http://www.cbs.dtu.

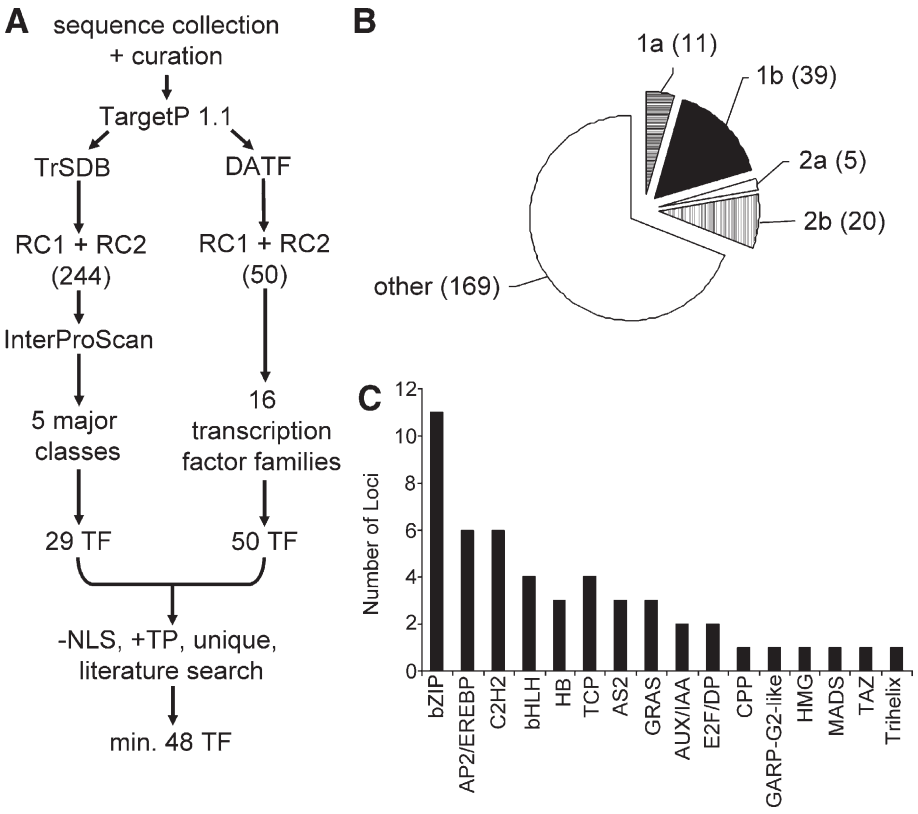

Fig. 1. Identification of candidate plastid transcription factors in TrSDB and DATF. A) Strategy for sequence search. Numbers in parentheses represent found sequences in the respective step of search. B) Number and functional category of putative plastid transcription factors identified from TrSDB analysed by InterProScan. InterProScan results were ordered hierarchically: 1a, sequences with known transcription factor domains including domains for interacting regulatory proteins. 1b, sequences with DNA-binding domains. 2b, sequences with domains related to translation. 2a, sequences with domains for RNA stabilisation. 3, sequences with domains for other known functions. For details see Supplementary Table 2. Numbers in parentheses are the number of found sequences in the respective category. C) Histogram of putative plastid transcription factors identified in DATF listed according to their transcription factor family. The *Y*-axis shows the number of found members of a given family.

dk/services/TargetP/) with no cut offs. Only sequences with a reliability class (RC) of 1 and 2 were used for further analysis. Redundant sequences within this data set were deleted. RC1 and RC2 sequences from TrSDB were scanned for known protein domains using InterProScan (http://www.ebi.ac.uk/InterProScan) before further analysis. All RC1 and RC2 sequences were tested for their predicted cellular localisation using the WoLF PSORT programme (Horton et al., 2006 http://wolfpsort.seq.cbrc.jp/). Furthermore, the sequences were tested for possible nuclear localisation signals (NLS) with the PredictNLS programme (http://cubic.bioc.columbia.edu/predictNLS/). DNA sequences of putative binding sites of identified transcription factor families were obtained also from the DATF database. Non-degenerated binding sites were compared with the chloroplast genome sequence of *Arabidopsis*. The plastome sequence was obtained from MIPS. Literature search was performed with Pubmed (http://www.ncbi.nlm.nih.gov/).

3. Results

In order to elucidate the possible presence, number and identity of nuclear encoded TFs targeted to plastids we analysed known and predicted *Arabidopsis* TF sequences for possible N-terminal chloroplast transit peptides using the TransitP programme (Emanuelsson et al., 2000). For this analysis we used protein sequences from two databases: 6084 protein sequences listed in TrSDB (Aguilar et al., 2002; Hermoso et al., 2004) and 1826 protein sequences from DATF (Guo et al., 2005). TrSDB provides putative TF sequences as predicted by TranScout and supported by homology within TrSDB proteomes resulting in a relative high number of possible candidates. Therefore this list may contain many proteins which do not function as TF. In DATF the definition of TFs was more strict and automated search was combined with manual curation of the sequences resulting in a much smaller number of possible TFs. Here it could be possible that some TFs might be lost. To test sequences as much as possible we used the data sets of both sources. The data sets overlap in part, however, they are not completely redundant, therefore the searches were performed independently from each other and the results were integrated at the end (Fig. 1A).

3.1. Analysis of TrSDB sequences

TrSDB provides a cellular localisation prediction based on the programme ProtLoc, but ProtLoc does not provide information about plastid localisation since it just distinguishes between

Table 1
Summary of nuclear encoded plastid transcription factors of highest probability

TF family + [binding site] and (gene)[a]	RC[b]	Locus	cTP[c]	Domains found with InterProScan[d]
Mov34, MPN, PAD-1	2	At1g10600	0.848	Mov34/MPN/PAD-1
Zinc finger	2	At1g05690	0.809	TAZ-type BTB/POZ KELCH-related protein
	2	At1g14580	0.739	C2H2-type major intrinsic protein (MIP)
	2	At1g26790	0.816	Dof-type
	2	At3g06660	0.839	HIT-type PAPA-1-like conserved region
	2	AT4g11910	0.817	C2H2-type
	1	At5g14010	0.962	C2H2-type
	2	At2g02070	0.836	C2H2-type
	2	At2g02080	0.888	C2H2-type MIP
	2	At5g43540	0.892	C2H2-type
	2	At5g54360	0.888	C2H2-type
Bromodomain	2	At1g73150	0.896	Bromodomain
	2	At3g54610	0.946	Bromodomain GCN5-related *N*-acetyltransferase
TPR repeat	2	At2g29670	0.791	TPR repeat
	1	At5g12430	0.98	Heat shock protein DnaJ TPR repeat protein prenyltransferase
MYB	1	At3g04450	0.88	Myb-like DNA-binding region, SHAQKYF class
	2	At5g60050	0.919	IMP dehydrogenase/GMP reductase Myb, DNA-binding BTB/POZ
AP2/EREBP [aGCCGCC CAACA	1	At1g21910	0.976	ETHRSPELEMNT AP2
+CACCTG gCAC[AG]N[AT]	1	At1g77640	0.956	ETHRSPELEMNT AP2
TcCC[ag]ANG[ct][ag]CCGAC] (rpoB)	1	At2g44940	0.921	ETHRSPELEMNT AP2
	1	At5g57390	0.979	ETHRSPELEMNT AP2
AS2	1	At3g13850	0.929	DUF260/LOB
	2	At1g07900	0.948	DUF260/LOB
	2	At1g06280	0.882	DUF260/LOB glycosyl hydrolase domain
AUX/IAA	1	At3g17600	0.979	Aux/IAA ARF dimerisation
bHLH [CACGGC/CACGAC CACGTG	1	At5g01310	0.982	Basic helix-loop-helix histidine triad (HIT) protein
CANNTG] (matk, trnK,	2	At3g06590	0.973	Basic helix-loop-helix
rbcL, trnl-01, rpl32,	2	At3g20640	0.854	Basic helix-loop-helix
trnl-02, rps2, ndhC, rps 19, trnQ, psbK, trnM, rpl16)				
bZIP [core: ACGT CCACGTGG	1	At1g06070	0.963	Basic-leucine zipper
TGACGTGG ATGACGTCAT]	1	At2g12900	0.979	Basic-leucine zipper
(rps7-04)	1	At2g12940	0.958	Basic-leucine zipper
	1	At2g13115	0.965	Basic-leucine zipper cAMP-response-element binding protein-related
	2	At2g21235	0.892	Basic-leucine zipper cAMP-response-element binding protein-related
CPP	2	At4g14770	0.892	Tesmin/TSO1-like, CXC
E2F/DP [c/gGCGCg/c]	2	At2g36010	0.875	Transcription factor E2F/dimerisation partner (TDP) Winged helix repressor DNA-binding
(trnR, trnA, psbB, orf77,	2	At5g03415	0.973	Transcription factor E2F/ dimerisation partner (TDP) winged helix repressor DNA-binding
rpl23-01, trnG, atpA, psbC, trnL, rbcL)				
GRAS	1	At3g54220	0.973	GRAS TF
	2	At1g55580	0.932	GRAS TF
	2	At4g00150	0.829	GRAS TF
HB	1	At5g11270	0.969	Homeobox
	2	At1g46480	0.951	Homeobox
	1	At2g29650	0.986	Major facilitator superfamily (MFS) homeobox protein, antennapedia type
	2	At3g03660	0.847	Homeobox
HMG	2	At2g34451	0.928	High mobility group box
TCP	1	At2g37000	0.941	TCP TF
	2	At1g35560	0.956	TCP TF
	2	At1g69690	0.896	TCP TF
	2	At1g72010	0.813	TCP TF

[a] TF class, in brackets: binding site information for a TF family provided by DATF, in parentheses: localisation of such binding sites in front of given plastid genes.
[b] Reliability class.
[c] Blasted or given gene locus.
[d] Functional domains as identified by InterProScan.

intracellular, extracellular, membrane, membrane-anchored and nuclear localisation. Our TransitP search revealed 826 sequences containing putative plastid target sequences, however, to increase the stringency we only regarded proteins in RC1 (116 proteins) and 2 (155 proteins) with the highest probabilities as likely candidates. To identify redundant information these 271 sequences were blasted against the *Arabidopsis* proteome by MIPS Blast (http://mips.gfs.de/prog/Athal/db/search/search-frame.html) and compared resulting in 244 unique sequences (Supplementary Table 1). The algorithms used for generating the TranScout list of 6084 putative or possible TFs in *Arabidopsis* contained degenerated consensus sequences. To obtain more

detailed data about the identified proteins we scanned the individual sequences in RC1 and RC2 for their domain structure using InterproScan (http://www.ebi.ac.uk/InterProScan). We could distinguish between five major classes of protein domains inside the 244 candidates: 1) DNA-binding domains involved in DNA replication or repair; 2) domains for TFs or regulators of TFs; 3) RNA-binding domains involved in transcript stabilisation; 4) RNA-binding domains involved in translation initiation and elongation and 5) domains involved in post-translational modifications, signal transduction, typical for enzymatic activities and conserved domains with unknown function (see Fig. 1B). Most of the 244 identified proteins contained several of these domains (see Supplementary Table 1). We focussed our further analysis on those proteins with clearly identified TF domains (29 proteins). The localisation prediction of these proteins has been cross-checked using the programme WoLF PSORT, which integrates several cellular localisation prediction parameters. Although it includes the TargetP weight matrix method for identification of plastid target sequences it predicted a nuclear localisation for many of the TFs. Using the programme PredictNLS, however, we identified a nuclear localisation sequence (NLS) in just a third of these 29 sequences. The reason for this contradiction is most probably a subtraction of 7.5 in the weight matrix score for cleavage site detection. We therefore regarded WoLF PSORT as not suitable for our specific question since it appears to weight the presence of a eukaryotic TF domain (and therefore a presumable nuclear localisation of the respective gene product) higher than the presence of a plastid-directing transit peptide. However, the programme provided valuable information for other localisations such as mitochondria or peroxisomes.

3.2. Analysis of DATF sequences

The TransitP search with the sequences from DATF resulted in 272 sequences exhibiting a possible plastid-directing transit peptide. In a literature search sequences for factors with experimentally determined cellular location other than the chloroplast were identified and removed from the list. From the remaining sequences 19 were classified into RC1 and 31 into RC2. These 50 sequences contained representatives for 16 different transcription factor families (Fig. 1C). WoLF PSORT predicted 16 sequences to be targeted to the plastids, 32 to the nucleus and 2 to mitochondria. PredictNLS found a NLS in only 11 of these 50 sequences (Supplementary Table 2).

3.3. Calculation of plastid-localised eukaryotic transcription factors

Considering the above mentioned facts we regarded all unique sequences with a transit peptide belonging to RC1 and RC2, no NLS, no mitochondrial location according to WoLF PSORT and no other known localisation based on experimental data as highly probable candidates to be chloroplast located transcription factors (Table 1). 48 sequences met these conditions. To our knowledge none of them has been reported before. Members of at least 15 different TF families are included in this list, some of their nucleus-located relatives have been analysed in detail earlier and conserved DNA-binding sites were reported. The DATF also provided those conserved DNA-binding motifs for some of the TF families. The existence of such binding sites on the chloroplast DNA would further support the idea of additional TFs within plastids. Therefore, all non-degenerated binding sites were searched on both strands of the plastid genome of *Arabidopsis*. In front of several plastid encoded genes we found putative binding sites for AP2/EREBP-, bHLH-, bZIP- and E2F/DP-family (Table 1) supporting that these factors act in plastids.

4. Discussion

Regulatory proteins such as TFs typically exist in very low concentrations preventing an easy biochemical characterisation. We therefore chose a bioinformatic approach to identify putative candidate genes for novel plastid TFs. Our study highly suggests the existence of many yet unidentified eukaryotic TFs targeted to plastids. The specificity of TargetP is approximately 69% suggesting a loss of a considerable part of really imported factors in our search (Emanuelsson et al., 2000). Prominent examples are the σ-factors which possess quite unusual transit peptides. We identified only SIG4 in our study while a total of six σ-factor genes have been described for *Arabidopsis* (see Section 1). In addition, to be more certain we considered only proteins classified to RC1 or 2 as probable candidates. Chloroplast proteins classified to RC3–5 therefore will be lost, too. All together we calculate from our data that at minimum 48 nuclear encoded regulatory TFs are imported into this organelle. However, this calculation is very conservative. If we take all theoretical losses into account then this number might be much higher and may increase up to 75–100. Furthermore, if we consider that eukaryotic TFs typically act in varying combinations of homo- and/or heteromeric complexes (respective scaffold domains are present in the identified genes) this would provide an enormous potential for the expression regulation of each single gene in the plastome. The existence of each predicted factor must be experimentally confirmed since it will largely affect our understanding of chloroplast transcription. First mass spectrometric analyses failed mainly because the small amounts of these regulatory proteins were masked by proteins of higher abundance such as ribosomal subunits. This has been observed even in protein fractions which were biochemically enriched in transcriptionally active proteins (Wagner and Pfannschmidt, unpublished results). Experiments are now in progress which aim to establish appropriate biochemical tools for the specific purification and identification of each single factor based on the present bioinformatic study.

Although no direct evidence for these TFs exists at present several observations support our assumptions indicating that our predictions are correct: i) the certainty of TransitP in general is about 85% indicating that 85% of all proteins predicted to be plastid-localised are real plastid proteins, ii) the predicted transit peptides of the 48 candidate genes were checked manually for characteristics of chloroplast transit peptides. All sequences started with MA or MAS and exhibited increased serine/

Table 2
Unusual *cis*-elements in known PEP promoters of higher plants

cis-element and promoter identity	Sequence	Conserved between monocots and dicots
−35/−10 PEP promoter (consensus)	TTGACa-17–19 nt–TATAAT-5–7 nt	Yes
BLRP[a] (psbD/psbC)	AAAGAAG, AAAGTAAG (AAG box) CAAAAAGG GTTCTTATCAAATCA A ATCCACCATAA (plastid GT box)	Yes
CDF2 binding site[b] (rrn)	AAGAGGCTCGTGGG	Yes
Region D[c] (psaA/psaB/rps14)	TAGAGAGATGGGAGATAGAA	Yes
TATA box-like element[d] (psbA)	TATATAA	Yes

[a] Blue-light responsive promoter, binding sites for AGF and PGTF (compare Introduction) (Hoffer and Christopher, 1997).
[b] Baeza et al. (1991).
[c] Cheng et al. (1997).
[d] Link and Langridge (1984).

threonine contents and only very few acidic amino acids which are typical properties of such transit peptides, iii) several mass spectrometric analyses of plastid RNA polymerases identified nuclear encoded regulatory factors in these complexes indicative for a change in subunit composition in the enzyme complex leading to a more eukaryotic-type enzyme, iv) even well-characterised TFs such as σ-factors were not identified in MS approaches so far indicating that plastid TFs exist in only substoichiometric amounts, v) a few eukaryotic DNA-binding elements typical for some of the predicted factors could be identified within chloroplast promoter sequences providing possible target genes (Table 1), vi) promoters of many plastid genes contain additional sites besides the typical PEP *cis*-elements which were tested experimentally to serve as recognition motifs for biochemically purified *trans*-factors such as AGF or CDF2 (compare Introduction, Table 2). Such motifs were distinct from all known prokaryotic-like promoters. Since they were found to be highly conserved among species this suggests that the interacting factors and the coupled regulatory response were developed early in evolution.

So far it was difficult to explain the high number of gene-specific transcription initiation sites observed in chloroplasts. The action of additional factors might be the solution for this problem and provides the potential to adapt transcription to a great variety of intra and extracellular signals. It would also provide an explanation for the weak developmental phenotypes observed in sigma knock-out mutants (see Section 1) since complex networks can compensate the loss of one component more easily than simple ones.

The integration of eukaryotic transcription factors into a prokaryotic expression machinery appears to be difficult to establish. So, what happened during endosymbiosis? An earlier hypothesis proposed a discontinuous evolution of the plastid genetic machinery in which the endosymbiont first lost its prokaryotic regulators because of the stable environment in the host cell followed by a second step in which eukaryotic factors are imported and integrated to provide an anterograde control over chloroplast gene expression (Sato, 2001). This idea does not consider that the endosymbiont still perceived environmental information despite its localisation within a host cell *via* the efficiency of the photosynthetic process which highly varied depending on the hosts' environment. We propose a different scenario which expands this first model and which also includes the impact of endosymbiosis on the development of the nuclear gene expression machinery (Fig. 2). In the beginning the plastid ancestor, an independent cyanobacterium (Fig. 2A), has been engulfed into the relatively stable environment of the host cell. Sensor kinases in the outer membrane might have lost their function, because immediate environmental changes sensed by these kinases were not present anymore. The genes of such 'unnecessary' kinases and their interacting response regulators therefore could be easily lost or transferred to the nucleus (Fig. 2B). Instead of these sensing systems signalling pathways from nucleus and cytosol into the endosymbiont must have evolved which reported the actual metabolic state of the host cell, the new 'environment' (Fig. 2C and see below). Since a mutual adaptation of the metabolism of host and endosymbiont was crucial for the establishment of a stable symbiosis metabolic signals might have been of great importance especially in the early phases of the endosymbiosis. However, the endosymbiont was not blind for the environment of the host cell. It additionally obtained important information about the environmental conditions *via* photosynthesis that exerted a specific control over the expression of genes such as those for photosynthesis components. Such control mechanisms still can be observed in living photosynthetic bacteria, many of them are under the control of redox-active photosynthetic components and are signalled *via* two-component systems (Pfannschmidt, 2003). We must assume that an easy transfer of genes to the nucleus could occur only as long as this endogenous signal transduction of the endosymbiont was not negatively affected since this would have had adverse consequences on photosynthesis. That implies that gene products that have an essential role in the endogenous signalling routes could be transferred to the nucleus only when the encoded product was either rerouted to the endosymbiont *via* a targeting sequence or when it was replaced by another factor with complementary function. The first case could be established *via* gene duplication and acquisition of target peptides for the nuclear copy (Fig. 2C). The σ-factors are a good example for this. This way provided a first nuclear control over the plastid gene expression. The factors identified by our bioinformatic search, however, might have been incorporated *via* the second way. A driving force for such a replacement is difficult to imagine since essential prokaryotic components could be lost only after substitution by the another eukaryotic component, but, on the other hand, such a substitution would occur only if there was a urgent need for it, i.e. after the loss of the prokaryotic component. A possible solution for this dilemma could be an interaction problem between the early signalling pathways from the host (which replaced the 'unnecessary' two-component systems for exogenous sensing) and the remaining prokaryotic machinery for the endogenous sensing of the endosymbiont. Such interactions

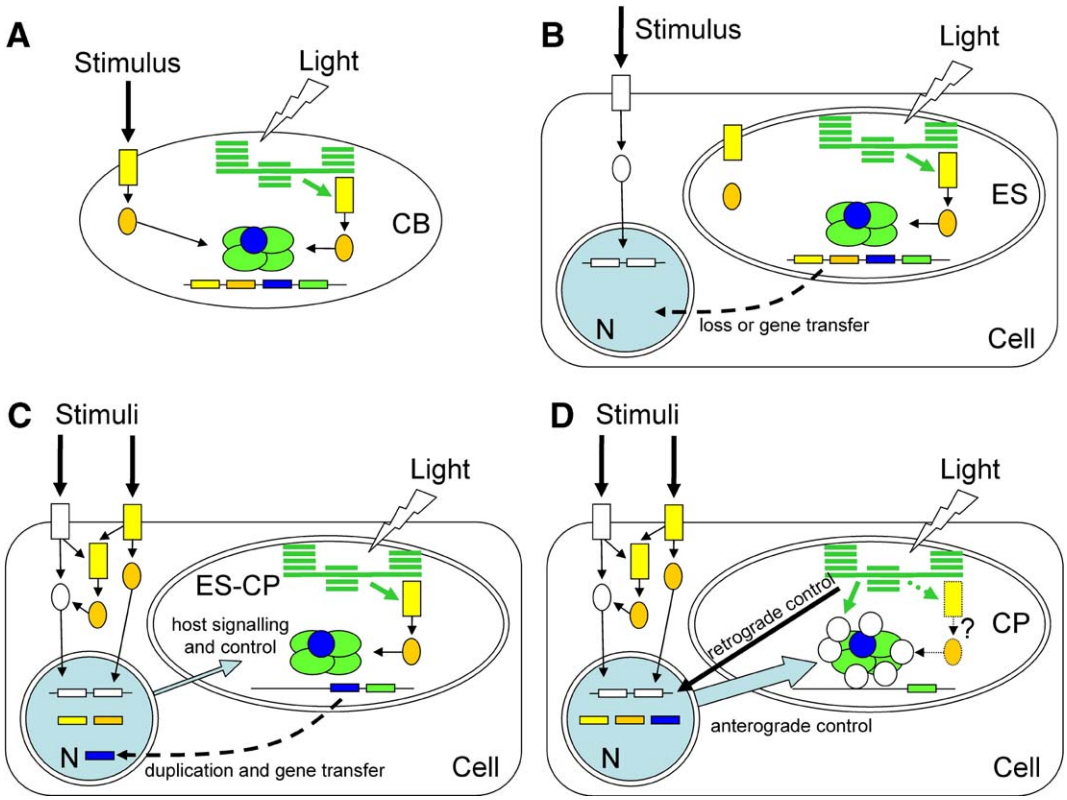

Fig. 2. Evolutionary model for the distribution of regulatory factors during endosymbiosis. A: A scheme of a cyanobacterium (CB) and its signalling and gene expression components. Perpendicular rectangles represent signalling components such as two-component sensor kinases (yellow) and response regulators (orange). Green circles represent subunits of the RNA polymerase, the blue circle represents a σ-factor. Horizontal bars in the same colour represent the corresponding genes. Thick black arrows are environmental signals which are sensed by membrane-located sensors. Thin black arrows represent the respective transduction of the environmental signal affecting gene expression. Endogenous signals originating from the thylakoid membranes (stacked green lines) are shown as green arrow. B: Engulfment of the endosymbiotic prokaryote (ES) into the eukaryotic host cell containing a nucleus (N). The two-component systems of ES do not perceive and transduce environmental signals within the host cell. The genes for them therefore are lost or transferred to the nucleus (broken arrow). Signals from photosynthesis are still existing. C: Ongoing endosymbiosis results in a transition from an endosymbiont toward a chloroplast (ES-CP). Two-component systems from the endosymbiont are used by the host either directly for signal transduction or indirectly by integrating the components into the eukaryotic signalling pathways (white symbols) which expands the ability to perceive environmental stimuli. The host cell sends first signals about its metabolic state to the ES-CP (thin blue arrow). Some genes are transferred to the nucleus after a duplication and the proteins are rerouted to the ES-CP allowing some host control of the expression within the ES-CP (thin blue arrow). D: Situation in a fully evolved chloroplast. The cell nucleus exerts a complex anterograde control over the chloroplast by sending various proteins of cyanobacterial (blue circle) or eukaryotic origin (white circles) which interact with the former bacterial RNA polymerase. These additional proteins allow the transduction of endogenous and exogenous signals in the chloroplast. The existence of bacterial two-component systems in higher plant chloroplasts is not clear yet (question mark). The communication between nucleus and chloroplast is completed with signals from the organelle toward the nucleus helping to adapt gene expression to the functional state of the chloroplast (retrograde control). (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

coordinate photosynthesis and e.g. nutrient demands in todays cyanobacteria and certainly were also necessary for the endosymbiont. In the chloroplasts of higher plants photosynthesis is perfectly coupled to the metabolic fluxes within the surrounding cell which is achieved by a extensive communication between the processes inside and outside of the chloroplast. Therefore the need for communication and coupling between endogenous and exogenous sensing routes within the endosymbiont may have provided the major driving force to replace prokaryotic regulators at all levels of gene expression. Beside this these eukaryotic factors gave the host cell further possibility to adapt and control processes within the endosymbiont to the requirements within the cytosol (anterograde control) and possibly provided the base for the tissue-type-specific or development-dependent plastid type which was developed in multicellular plants (Fig. 2D). If all prokaryotic signalling components and TFs were replaced by eukaryotic components is not clear yet. Under our stringent search conditions we found no two-component system factors imported into the higher plant organelle. Nevertheless, some two-component system like proteins were identified recently in the *Arabidopsis* nuclear genome which exhibit N-terminal extensions with a possible transit peptide function (Forsberg et al., 2001; Oelmüller et al., 2001) or which are fused to other protein domains (Weber et al., 2006).

In summary in our evolutionary model gene transfer starts with a probably short phase in the beginning of endosymbiosis in which unnecessary prokaryotic gene expression regulators are lost. This phase then passed over into a long and continuous phase in which the remaining prokaryotic systems for processing of endogenous photosynthetic signals are step by step coupled to the eukaryotic-type components for the exogenous signalling

from the host cell toward the endosymbiont. As a result the basic prokaryotic gene expression machinery of todays chloroplasts was covered by a coat of eukaryotic protein components which provide a functional interface for the communication between the organelle and the rest of the cell.

The evolutionary processes described above had also a second effect since the mixing of eukaryotic and prokaryotic regulators of gene expression was not uni-directional. Many genes lost from the endosymbiont in the early phase were absorbed by the nucleus. It integrated the additional functions of the encoded components to expand its own range in sensing and processing of environmental stimuli (Fig. 2C). Indicative for this is the high number of two-component systems found in higher plants while none or only very few are present in non-photosynthetic eukaryotes such as mammals or fungi (Chang and Stewart, 1998). In this context it is interesting to note that plants in turn have a limited G-protein based signalling machinery in comparison to other eukaryotes (Jones and Assmann, 2004). Since endosymbiosis in the green line occurred very early in evolution (far before the development of plants) (Delwiche, 1999) the early integration of two-component systems could be a possible reason why a strong diversification of G-protein based signalling network became unnecessary.

Our model suggests the evolutionary establishment of combined pro- and eukaryotic gene expression machineries in nucleus and chloroplasts which supports and supplements the idea of the plant genetic system as an integrated system as proposed earlier (Herrmann et al., 2003).

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.gene.2006.06.022.

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VII

Wagner, R., Fischer, W., Leister, D. and Pfannschmidt, T. **(2006)**. "The role of long-term light quality acclimation in photosynthetic performance of *Arabidopsis thaliana*." Manuskript in Vorbereitung für Plant Physiology.

In diesem Manuskript werden verschiedene physiologische Leistungen von *A. thaliana* nach Akklimation an PSI- und PSII-Licht wiedergegeben. Mutanten mit Defizienzen in der Kurz- und/oder der Langzeitantwort werden zum Vergleich herangezogen, um die Bedeutung photosynthetischer Lichtqualitäts-Akklimation für die einzelne Pflanze zu zeigen.

Running head

Long-term light quality acclimation of photosynthesis

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Title

The role of long-term light quality acclimation in photosynthetic performance of *Arabidopsis thaliana*

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Abstract

Long-term acclimation of higher plants to varying light qualities involves a re-adjustment of photosystem stoichiometry which is thought to increase the photosynthetic yield. To quantify this effect *Arabidopsis thaliana* was acclimated to light sources which preferentially excite photosystem I or photosystem II (PSI- or PSII-light). Characteristic differences are a higher chlorophyll (Chl) fluorescence parameter Fs/Fm and a higher degree of thylakoid membrane stacking in PSI-light acclimated plants (PSI plants) when compared to PSII-light acclimated plants (PSII plants). Detailed analyses of PSII Chl fluorescence under growth light conditions revealed only minor differences between these plants with respect to Fs/Fm, 1-qP and the effective quantum yield of PSII. In general, however, the plastoquinone pool appeared more oxidised under PSI- than under PSII-light. P700 activity measurements using absorbance changes at 830 nm indicated fully functional PSI in all of the differentially acclimated plants. Furthermore, light stress experiments revealed no effect of the long-term response (LTR) on the process of photoinhibition and the recovery from it. To uncover the beneficial potential of the LTR we compared wild-type (WT) plants with the state transition mutant *stn7* which lacks also the LTR. *Stn7* mutants revealed clear differences in PSII Chl fluorescence resulting in a decreased effective quantum yield. Other state transition mutants implicate that this is neither due to the LHCII migration nor to the pure existence of LHCII pointing to the STN7 kinase as the central regulator of LTR and state transition. When testing the seed production under fluctuating light quality conditions *w*ild-type plants produced almost the double amount of seeds when shifted permanently between PSI- and PSII-light. Despite the hardly detectable differences in WT photosynthetic performance the LTR has a pronounced positive effect on plant fitness in addition to state transitions.

Introduction

The light environment of photosynthetic organisms can highly vary in intensity and quality. In addition, such variations can occur on a broad time scale which ranges from seconds and minutes up to hours, days and even seasonal changes. The variations directly affect the efficiency of the photosynthetic light reaction and therefore ask for a high flexibility of plants in acclimating the photosynthetic process to such conditions. Most known acclimation responses act at the molecular level by modifying photosynthetic components at the post-translational level or by modulating their expression or assembly (Aro and Andersson, 2001; Blankenship, 2002; Walters, 2005).

Compared to the full sun spectrum the visible spectrum in dense plant populations is depleted in blue and red wavelengths and enriched in far-red wavelengths. This results in an imbalance of excitation energy distribution between photosystemI (PS) I and PSII since PSII absorbs less photons than PSI under these conditions. Since the two photosystems work electrochemically in series each imbalance has a direct effect on the efficiency of the electron flow. In the short-term plants acclimate to such a light quality gradient by state transitions, a process in which the relative antenna size between the two photosystems is changed within minutes (short-term response, STR) (Allen and Forsberg, 2001; Haldrup et al., 2001; Wollman, 2001). This is achieved through phosphorylation of the mobile light harvesting complex of PSII (LHCII) by a redox-sensitive kinase which is activated when the plastoquinone (PQ) pool becomes reduced (Allen et al., 1981). Recently, in *Chlamydomonas* and *Arabidopsis* mutants were identified which lack the thylakoid associated kinases STT7 and STN7, respectively (Depege et al., 2003; Bellafiore et al., 2005). These mutants are defective in LHCII phosphorylation and state transitions pointing to an essential role of the two kinases in these processes. The photosynthetic properties of the *stn7* mutant have been analysed in more detail recently confirming the proposed role of STN7 in the state transition process (Tikkanen et al., 2006). In *Arabidopsis* also other state-transition deficient mutants have been described which lack either PSI subunits such as PSAH (Lunde et al., 2000) or PSAE1 (Pesaresi et al., 2002) or the LHCII itself due to down-regulation of the essential LHCII subunit Lhcb1 and Lhcb2 (Andersson et al., 2003). Biochemical analyses on spinach as well as genetic manipulations in *Chlamydomonas rheinhardtii* demonstrated that the PQH_2 binding site within the cytochrome b_6f (cyt b_6f) complex

plays a decisive role in the detection of the PQ redox state and the activation of the LHCII kinase (Vener et al., 1997; Zito et al., 1999). On the base of these observations working models hypothesise that the PQ redox state is transmitted *via* conformational changes within the Rieske protein and the cytochrome f protein (Wollman, 2001). The multiplicity of regulatory sites involved in the state transition process indicates that the photosynthetic machinery as a whole contribute to the regulation of this acclimatory response suggesting a complex regulatory network acting on the post-translational modification of the LHCII.

In addition to the STR a second response can be observed which acts on a longer time scale (long-term response, LTR). In this response the stoichiometry of the photosystems is re-adjusted within hours or days by actively changing the relative number of the two photosystems in favour of the rate-limiting one (Melis, 1991; Kim et al., 1993; Walters and Horton, 1994; Allen, 1995; Anderson et al., 1995; Fujita, 1997). This supramolecular re-organisation of the thylakoid membrane complexes requires a complex control of degradation and assembly of the photosystems as well as a control over the expression of the genes encoding these respective subunits. Studies on gene expression in pea and spinach revealed specific effects on chloroplast gene expression acting mainly on post-transcriptional level (Glick et al., 1986; Deng et al., 1989). Further studies on *Sinapis* and pea indicated a transcriptional control of the photosystem reaction centre core protein genes *psaA* and *psbA* (Pfannschmidt et al., 1999; Pfannschmidt et al., 1999; Tullberg et al., 2000). Additional experiments manipulating the redox state of the electron transport chain by either light quality or by inhibitors indicated that the redox state of the PQ pool is the decisive regulatory factor. Comparable results were obtained in cyanobacteria and the red alga *Chlamydobotrys stellata* (Alfonso et al., 1999; Kovacs et al., 2000; Li and Sherman, 2000) indicating that this mechanism of PQ redox control represents a highly conserved regulatory process (Pfannschmidt, 2003) .

STR and LTR work on different time-scales but in the same functional direction which is a re-distribution of the absorbed excitation energy in favour for the rate-limiting system. However, the degree to which both processes improve the light utilisation of higher plants is still discussed. State transitions in green algae like *Chlamydomonas rheinhardtii* involve a migration of around 80% of the LHCII between the two photosystems (Allen, 1992; Delosme et al., 1996). In contrast, in higher plants only 15-20% of the LHCII are shifted (Allen, 1992). In the alga the state

transition is equivalent to a metabolic re-programming which shifts the cell between two very different metabolic states (Wollman, 2001). In state 1 (LHCII bound at PSII) the photosynthetic apparatus performs linear electron flow and CO_2 fixation in the Calvin-Benson cycle while in state 2 (LHCII bound to PSI) cyclic electron flow around PSI is preferred which converts the photosynthetic apparatus into an ATP generator. The smaller fraction of mobile LHCII in higher plants suggests a less dynamical change in structure and function of the photosynthetic apparatus in comparison to algae. This appears to be confirmed by observations on *Arabidopsis* mutants which are defect in state transitions. PSAH and PSAL deficient plants exhibited only slightly reduced efficiency in PSII photochemistry, i.e. *psaH* mutants revealed a 8-14% reduced oxygen evolution (Lunde et al., 2003). The *Arabidopsis* mutant *stn7* revealed slightly less fresh and dry weight in comparison to wild-type when grown under alternating low and high white light (Bellafiore et al., 2005). In general, all state transition mutants described so far are able to compensate for the defect to a high degree.

Interestingly the *stn7* mutant is also devoid of the LTR (Bonardi et al., 2005). The physiological effect of the LTR has been studied until now only in one study where PSI- or PSII-light acclimated pea leaves were investigated (Chow et al., 1990). The study focussed on the determination of the photosynthetic quantum yield expressed as mol of O_2 evolved per mol of photons absorbed. The yield of PSII plant leaves under PSII light was found to be 21% better than that of PSI plant leaves while that of PSI plant leaves under PSI-light was 19% better than that of PSII plant leaves. Other photosynthetic parameters as well as the consequences for plant growth and development, however, have not been determined. Therefore, the importance of the role of the LTR and the degree of its positive effect as well as its functional relation to state transitions is still largely unknown.

Here we describe for the first time the positive effects of the LTR on various photosynthetic parameters in *Arabidopsis.* By analysing different state transition mutants including the LTR mutant *stn7* we analysed the benefit of the LTR in comparison to the state transition and define the functional relation of these two acclimation responses in *Arabidopsis thaliana*.

Results

Phenotypic effects of LTR at leaf and cell level

Arabidopsis seedlings were grown under PSI or PSII lights to induce long-term light quality acclimation as described earlier (Fey et al., 2005). In normal white light no major phenotypic variations between the differentially acclimated plants became visible with the exception that PSI-plants tend to erect the leaves which is most probably a beginning shade avoidance reaction induced by the far-red wavelengths in the PSI light source. 2D Chl fluorescence imaging of the same plants revealed distinct differences in the Chl fluorescence parameter F_s/F_m (Fig. 1A). As in earlier measurements with a PAM fluorometer (Sherameti et al., 2002) PSI plants exhibit a higher F_s/F_m value than PSII plants. This relation was reversed when the plants were shifted to the respective other light source (PSI-II and PSII-I plants) indicating the reversibility of this reaction, an important criterion for a true acclimation response. The 2D imaging also revealed that Chl fluorescence slightly varied among leaves of individual *Arabidopsis* plants grown under the same condition, however, integration of the fluorescence signal over the complete leaf surface revealed statistically significant differences between the four growth conditions. This change in the F_s/F_m value was found recently to be a non-invasive indicator for the LTR in *Arabidopsis* (Bonardi et al., 2005; Fey et al., 2005). Electron micrographs of chloroplasts from PSI and PSII plants showed that the light quality acclimation involved massive changes in the structure of the thylakoid membrane (Fig. 1 B). PSII plants exhibited evenly distributed stroma and grana lamellae while in PSI plants the thylakoid membranes are organised in very large grana stacks which are connected by a small number of stroma thylakoids. The total thylakoid volume did not seem to be changed, therefore, the extreme stacking of the membranes generated a large membrane-free space in the chloroplasts. Furthermore, chloroplasts of PSII plants always contained several starch granules while no or only little starch could be found in chloroplasts from PSI plants. Instead, an increase in plastoglobuli number and/or size could be found. These structural data are consistent with earlier results obtained with other plants (pea, spinach, *Phaseolus*, *Atriplex*) under comparable growth conditions (Melis and Harvey, 1981; Deng et al., 1989).

LTR effects on photosystem efficiencies

The observed structural changes in the thylakoid membranes which occurred during the LTR are accompanied by changes in the supramolecular structure of the photosynthetic apparatus itself, i.e. in the stoichiometry of the photosystems (Fey et al., 2005). These structural changes are discussed to be the reason for the distinct differences in various Chl fluorescence parameters such as Fs/Fm or 1-qP which reflect the counterbalancing effect of the LTR on the excitation energy imbalance induced by the light quality gradients. However, such differences represent only indirect indications for the physiological function of the LTR since the respective measurements were performed with a standardized red light source as electron driving force which is similar to the PSII-light source concerning its spectral quality. To uncover the real beneficial effect of the LTR on photosynthetic efficiency we measured the same typical Chl fluorescence parameters with the growth light sources as actinic light (Table 1 and Fig. 2). In general, the Chl fluorescence under PSII-light was higher than under PSI-light which was not unexpected since the main part of Chl fluorescence at room temperature originates from PSII. The values for Fs/Fm, 1-qP and ΦPSII under the PSII-light and especially the characteristic differences induced by the various acclimation regimes were comparable to those from earlier measurements with a standard actinic red light diode (Sherameti et al., 2002). Fs/Fm was higher in PSI- and PSII-I plants than in PSII and PSI-II plants while 1-qP values indicated a higher reduction state of PQ in PSI and PSII-I plants than in PSII and PSI-II plants. This confirms the similarities in the excitation properties of the PSII-light and the actinic red light diode usually used. With the PSI-light source as actinic light, however, we found no significant differences for Fs/Fm in the differentially acclimated plants. The 1-qP values indicated that in all types of plants the PQ pool was more oxidised under PSI-light than under PSII-light. The PSI-light had almost the same effect as the strong oxidising far-red light diode (Fig. 2) which was used to determine the F_0' at the end of the measurement (compare Materials and Methods). Only the ΦPSII value was higher under PSI-light, which is caused exactly by the same fact that F_t under these conditions was almost at the same level as F_0'. All together these measurements indicate that plants under PSI-light indeed have a more oxidised electron transport chain than under PSII-light. Potential beneficial effects induced by the LTR were not detectable with the PSI-light as actinic light source.

All Chl fluorescence parameters tested above depend on the function of PSII. To test the functionality of PSI we measured relative P700 absorbance changes at 830 nm (Fig. 3) (Klughammer and Schreiber, 1998). Plants from all four acclimation regimes exhibited functional PSI with a typical wild-type-like redox behaviour. In some measurements we noticed a slight increase in the ΔA_{P700} value in PSI-II plants in comparison to the control plants grown in PSI light. In contrast, some PSII-I plants showed a decrease of the ΔA_{P700} value in comparison to the PSII control plants. This could be interpreted as a higher transport potential for electrons but is not a representative parameter for the number of PSI particles in the differentially acclimated plants.

LTR and light stress

It has been suggested that state transitions in *Chlamydomonas* may play also a role in the protection from photoinhibition (Finazzi et al., 2001). In *Arabidopsis* that appeared to be not the case (Lunde et al., 2003). However, to test if maybe the LTR has an effect on photoinhibition we illuminated differentially acclimated *Arabidopsis* plants with 1300 µE for 1 hour and determined the F_v/F_m value. The high-light treatment resulted in an expected decrease of the F_v/F_m value which was most pronounced in the PSI-light acclimated plants. After the high-light treatment plants were shifted to weak white light and the recovery was determined by detecting the F_v/F_m at several time points. In all cases the F_v/F_m value returned to almost normal levels within 28 hours. Thus, the LTR appears to make PSI plants slightly more susceptible to photoinhibition, but the repair cycle was not affected.

Non-photochemical quenching (NPQ) is one major protection mechanism which dissipates excess excitation energy (Niyogi, 2000). In earlier measurements we never observed any significant effect of the LTR on this process (data not shown), however, the measurement of NPQ under the growth light sources resulted in significant differences (Table 2). NPQ was lower always in those plants which had been acclimated to the respective actinic light quality, i.e. NPQ was lower in PSII and PSI-II plants under PSII-light when compared with PSI- or PSII-I plants and *vice versa*. The protection of photosynthesis by NPQ is known to reduce its quantum yield since light energy is dissipated as heat and not used for photochemical work (Niyogi et al., 2005). Precisely this can be observed for PSI and PSII-I plants under PSII light (Table 1) where NPQ is higher and the resulting photosynthetic efficiency is lowered.

The opposite behaviour of PSII- and PSI-II plants under PSI-light could be also observed in tendency, but the differences were not statistically significant. Thus, the acclimation to a specific light quality results in a re-direction of energy flow from non-photochemical to photochemical processes. It should be noted that our measurements reflect the actual NPQ in the specific environment and not the maximal capacity for NPQ.

Analysis of the functional relationship of LTR and state transitions

The state transition mutant *stn7* has been shown to be defective in the LTR. This provides a tool to study the impact of the LTR on photosynthetic efficiencies. We performed our light quality acclimation programmes with this mutant and determined the same photosynthetic parameters as with wild-type (Table 3; compare Table 1 and Fig. 2). As in wild-type the Chl fluorescence under PSII-light was generally higher than under PSI-light, however the values for Fs/Fm, 1-qP and ΦPSII differed from the wild-type values. Fs/Fm under PSII-light was higher and exhibited no significant differences between the differentially acclimated plants which is consistent with our earlier results (Bonardi et al., 2005). Such higher values were even observed under PSI-light. Consequently, we observed much higher 1-qP values in *stn7* indicating a higher reduction state of PQ in *stn7* under all growth light regimes. The 1-qP values under PSI-light indicated that in all types of plants the PQ pool was more oxidised under PSI-light than under PSII-light, however, its oxidising effect was weaker than in wild-type. The ΦPSII value both under PSI- and PSII-light was decreased in comparison to wild-type indicating that the photosynthetic efficiency of *stn7* under the growth lights was clearly reduced. This demonstrates that the loss of LTR has negative effects on the photosynthetic performance of the mutants.

In wild-type the LTR had an influence on NPQ, i.e. it decreased it (Table 2) resulting in higher effective quantum yields. We observed the same tendencies in the *stn7* mutant, however, these changes were not statistically significant and, in addition, values for NPQ under all conditions were always lower than in wild-type. The loss of LTR thus also reduced the ability of the mutant for energy dissipation by heat.

These effects of the LTR defect of *stn7* led us to the question if these effects are due to the loss of kinase activity or to the loss of the LHCII shifts. To figure out if the phosphorylation state of LHCII or its migration is responsible for triggering the

LTR we analysed two other state transition mutants with different defects. *psaE1-1* lacks the PSI subunit E1 and is locked in state 2 since the LHCII is not able to leave PSI anymore forming a stable LHCII-PSI supercomplex (Pesaresi et al., 2002). *asLhcb2-12* is silenced in *Lhcb2* resulting in a LHCII-less plant (Andersson et al., 2003). We performed our standard growth regimes under the PSI- and PSII-light sources and tested the ability of the mutants for long-term acclimation to the different light qualities. No significant differences relating to F_s/F_m, 1-qP and Chl *a/b* in comparison to wild-type control plants grown on the same plates could be detected (Fig. 5). Since both mutants exhibited the typical changes in Chl fluorescence and Chl *a/b* ratio we conclude that neither the phosphorylation state of LHCII nor its migration are involved in the regulation of the LTR. Furthermore, the clear changes in the Fs/Fm values observed with the *asLhcb2-12* mutant indicate that this Chl fluorescence parameter is not influenced by the external antenna at all demonstrating that the state transition does not significantly contribute to the respective changes. This suggests that the loss of the STN7 kinase activity is responsible for the loss of the LTR.

To understand the ecophysiological importance of STR and LTR, respectively, we tried to determine effects on the fitness of differentially acclimated *Arabidopsis* plants. Recently, it was reported that seed production is a good parameter for assessing productivity and hence fitness of *Arabidopsis* (Kulheim et al., 2002). We therefore grew wild-type plants in PSI- or PSII-light until they flowered in order to test if the plants are able to produce seeds under these very adverse conditions. In comparison to white-light grown control plants both populations showed a retarded development, but they survived, flowered and produced viable seeds. The plants were able to produce ~ 5 - 10% of seed material of the control plants (Fig. 6A) demonstrating that *Arabidopsis* is able to use the light energy of these light sources for growth despite the uneven excitation of PSI and PSII. To assess the individual roles of the two acclimation responses we performed light shift experiments with *stn7*, *stn7/stn8*, *stn8* mutants and wild-type. The plant populations were grown in parallel under permanently changing PSI- and PSII-light conditions. To test the importance of the LTR plants were shifted every 2 - 3 days between the light sources, to test the role of the STR plants were shifted every 20 minutes. The respective time frames were adjusted to the corresponding ranges necessary for plants to perform the typical LTR or STR, respectively. Populations of both, mutants

and wild-type plants showed growth, flowering and seed development under both growth regimes. Germination tests confirmed that the collected seed material was completely viable (data not shown). The development of the plants under the short-term light shifts was delayed in comparison to those grown under long-term shifts including the development the florescence. For a full generation cycle the population under short-term shifts needed 19 weeks in contrast to 14 weeks for the long-term shift plants. The general phenotype of both populations including the *stn7* mutants revealed no obvious differences. Due to the permanent illumination rosettes were relatively small (leaf number and diameter) with leaves exhibiting long petioles and small oval *laminae* of 0.5 up to 1.5 cm in length. The typical inflorescences were between 40 and 45 cm high, branched and developed normal numbers of flowers. However, many of the flowers did not develop to a silique or the siliques were small and sterile. The *stn7* mutant produced around 50 % less seeds than the wild-type under the long-term shifts while *stn8* produced same or slightly more amount of seeds (Fig. 6B). The double mutant *stn7/stn8* behaved like the *stn7* single mutant. This is consistent with earlier observations (Bonardi et al., 2005) that the STN7 kinase is involved in the LTR while the STN8 kinase is not necessary. Under short-term light shifts *stn7* produced around 40 % of the seed numbers of wild-type controls pointing to the importance of state transitions under fast changing illumination conditions (Fig. 6C). It is interesting to note that plants grown under short-term light shifts produced significantly smaller amounts of seeds than those grown under long-term light shifts regardless of being mutant or wild-type. Taken together these fitness experiments provide conclusive evidence that both STR and LTR are very important for *Arabidopsis* to acclimate the photosynthetic process to varying light qualities and that the lack of these responses provide an evolutionary disadvantage for the survival of the species.

Discussion

Arabidopsis as model organism is widely used in many fields of molecular plant biology, however, in photosynthesis research it has been used only hesitantly. Major drawbacks of this plant are the slow growth exacerbating the yield of high amounts of material and the difficulty to obtain intact chloroplasts of sufficient quality, both important properties for many studies in photosynthesis research. Nevertheless, the

unique genetic advantages convinced several groups in the last decade to use this plant to tackle important open questions in photosynthesis (Leister, 2003). Mutant screens or analysis of gene-specific knock-out mutants uncovered novel protein components with important functions mainly in the regulation and environmental acclimation of the photosynthetic light reaction. Prominent examples for this development are the discovery and characterisation of regulatory proteins such as PsbS (Li et al., 2000), PsaH (Lunde et al., 2000), Stn7 (Bellafiore et al., 2005) and Stn8 (Bonardi et al., 2005). Beside these studies, the number of detailed physiological analyses of photosynthetic properties of this plant is still relatively small. Much pioneering work has been performed by Walters and colleagues who systematically analysed the acclimation abilities of *Arabidopsis* to varying illumination conditions in detail (Walters and Horton, 1994, 1995; Walters et al., 1999; Bailey et al., 2001). These studies clearly demonstrated the usefulness of this small model plant in the analysis of photosynthetic acclimation responses. A further important step forward was the use of this plant in eco-physiological studies in which the molecular analysis was combined with the determination of the plant fitness under natural conditions (Kulheim et al., 2002). This study showed that the high number of seeds could be easily used to analyse to which degree the plant is able to deal with a given environmental situation. Our study here is a combination from all of these approaches integrating physiological measurements with mutant studies and fitness experiments and complements a recent study on the state transition properties of *Arabidopsis* wild-type and the *stn7* mutant (Tikkanen et al., 2006).

The LTR of *Arabidopsis* to the different light qualities involves typical changes in the Chl fluorescence which are based on characteristic structural re-arrangements in the thylakoid membranes. The strong stacking within PSI chloroplasts (Fig. 1) is consistent with current models on LHCII phosphorylation and its influence on grana structure (Allen and Forsberg, 2001). Under PQ oxidising conditions which are induced by PSI light (compare Table 1) the LHCII kinase should be inactive and the photosynthetic apparatus should be in state 1 resulting in higher grana stacks as observed in many higher plants (see introduction). Thus, the studies on *Arabidopsis* presented here are representative for understanding light quality acclimation of higher plants in a general term rather than being a case study.

The most important open question is that for the functional role of light quality acclimation responses in the physiology of plants. Recent studies reported only minor

beneficial effects of STR as well as LTR (compare introduction). Our Chl fluorescence measurements on *Arabidopsis* here (Fig. 1) and earlier (Sherameti et al., 2002) are consistent with this, however, in all these experiments the measured effects reflect beneficial effects only indirectly since the measurements were not performed under the growth light conditions. In order to detect the "true" improvement of the LTR we measured the differentially acclimated plants in their growth cabinets using the growth light as actinic light instead of the red light source usually used in Chl fluorescence measurements. The decisive differences to earlier experiments are i) that photosynthetic activities of PSI or PSII were determined directly in the environment to which the plants have been acclimated and ii) that these lights are much weaker then the actinic red light usually used (~ 25 versus ~ 90 µE photons). Under PSII light we obtained comparable results to our earlier experiments with regard to the qualitative changes (Sherameti et al., 2002). The weaker intensity of the PSII light, however, resulted in a decrease of the detected fluorescence signal by roughly 50 % indicating that the strength of the actinic light has a clear impact on the results of these measurements which should not be neglected. Under PSI light the fluorescence signal further declines indicating the oxidising effect of this growth light source. Since the detected signal was near the F_0' qualitative changes were not observable anymore.

Both, PSI- and PSII-plants represent low-light acclimated plants. Therefore, it was not surprising that all plants revealed photoinhibitory effects when exposed to a strong white light. The slightly stronger effect on PSI-plants is consistent with a higher PSII/PSI ratio in these plants compared to PSII-plants leading to faster saturation of the light reaction. On the other hand, the kinetic of the recovery was not affected suggesting that the LTR in general is not interfering with the repair cycle.

Most Chl fluorescence data obtained here exhibit some differences between the differentially acclimated plants, nevertheless, these differences were rather small and it was difficult to evaluate the importance of such tiny changes for a plants life in a quantitative way. We therefore decided to determine the plant fitness under the different light qualities *via* calculating the number of produced seeds. We used the LTR mutant *stn7* as a negative reference to visualise the acclimation ability of wild-type plants. The studies on the two state transition mutants *asLhcb2* and ∆*psaE1* clearly indicate that a defect in LHCII migration is not necessarily correlated with a loss of the LTR. This points to *stn7* as the presently only mutant exhibiting a defect in

the long-term acclimation to light quality suggesting that the STN7 kinase regulates STR and LTR in a combined manner. Initial growth experiments revealed that the plants are able to perform a full generation cycle under these light sources (Fig. 6A) indicating that they can survive even under the limited light conditions. However, the decrease in seed production in comparison to the white light grown control plants indicate that the conditions are still not ideal. Thus, the acclimation cannot fully compensate for the imbalance in excitation energy created by the spectral quality gradients of the light sources. This shows that our light system is extreme enough to force the full physiological range of the plants acclimation ability, but it is not too extreme to kill them.

One important property of acclimation responses is their dynamical reversibility. To test the physiological impact we performed light shift experiments which target to these properties. Wild-type plants grown under short-term shift conditions exhibited about a 2 fold higher seed production than the state transition mutants *stn7* and *stn7/stn8* while the PSII kinase mutant *stn8* revealed no negative effects. This reveals a clear advantage of the state transition for the survival of the plant and demonstrates the importance of this acclimation response. Until now there was still a debate on the physiological impact of state transitions in higher plants and earlier studies reported only minor effects on size (Bellafiore et al., 2005) or even no visible phenotypes (Tikkanen et al., 2006). Thus, our data clearly indicate for the first time that the LHCII shifts during the state transition, even if this accounts only for a minor fraction of the antenna, are of great importance for the plant to harvest extra light under limiting conditions. This light energy then is used to produce more seeds and to increase the survival rate of the species.

The physiological impact of the LTR in higher plants was even more enigmatic than that for the state transition since until now there was only one report demonstrating a certain beneficial effect on photosynthetic yield (Chow et al., 1990). Our experiment under the long-term light shifts clearly demonstrates that wild-type plants which can acclimate to such light quality shifts produce a 2 fold higher number of seeds than the *stn7* mutant. Thus, the benefit of the LTR is in the same order of magnitude as the state transition. Therefore, the energy-consuming changes in gene expression under persistent light quality gradients are profitable. This reveals an impressive acclimation ability of *Arabidopsis* both under short-term and long-term light quality shifts which covers a broad time scale of light fluctuations.

Interestingly wild-type and mutant plants grown under long-term shifts produced clearly more seeds than under short-term shifts. Furthermore the plants under the 20 min light shifts developed more slowly. This is somehow surprising since plants of the same age should have perceived the same number of light quanta and hence light energy in the growth cabinets. Since the mutants are affected in the same way as wild-type plants this suggests that the difference is not related to the different acclimation responses. One possibility might be that the permanent short-term light quality shifts interfere with an endogenous (circadian?) rhythm of the plants which is not the case under the long-term light shifts. Further experiments are needed to understand this phenomenon.

Our data indicate that the small differences in photosynthetic performance induced by STR and LTR result in very effective acclimation to the residing light condition which in turn enable the plant to produce enough seeds for the next generation. This represents a clear evolutionary selection pressure on the maintenance and optimisation of acclimation responses in higher plants.

Materials and Methods

Plant material

Arabidopsis thaliana (L.) Heynh., ecotype C24 was used as wild-type (WT) throughout the study. As reference for T-DNA insertion lines from the Salk Institute collection *Arabidopsis thaliana* ecotype Columbia 0 (Col-0) was used. State transition mutants analysed were *stn7* (Bellafiore et al., 2005; Bonardi et al., 2005), *psaE1-1* (Pesaresi et al., 2002) and *asLhcb2-12* (Andersson et al., 2003).

Growth conditions and light treatments

Plants were grown in temperature controlled growth chambers at 20°C under continuous light. *Arabidopsis* seeds were sown either sterile on MS medium containing 2 % sucrose or on earth mixed with vermiculite. The density of seeds was adjusted in such a way that 16-day-old plants did not shadow each other. After two days at 4°C plants were first grown for 10 days under white light provided by 30 W white stripe lamps (OSRAM, München, Germany) with a photon flux density of 60 µE. Plants were then acclimated to PSI- or PSII-light for 6 days (PSI or PSII plants) or they were first acclimated to one light source for 2 days followed by 4 days in the respective other light source (PSI-II or PSII-I plants). PSI- and PSII-light sources have been described earlier in detail (Fey et al., 2005).

Electron microscopy

For electron microscopy, small stripes from the middle portion of leaves were fixed by combination of 2.5% (v/v) glutaraldehyde and 1% formaldehyde (v/v) in 50 mM cacodylate buffer (pH 7.0) for 2h at room temperature. After thorough rinsing in the above buffer, post-fixation was carried out in cold 1% (w/v) osmium tetroxide in the same buffer for 1h at 4°C. Following dehydration in graded acetone series, the samples were embedded in Spurr's resin (Spurr, 1969). Sections stained with uranyl acetate and lead citrate (Reynolds, 1963), then examined in an Zeiss EM 900 electron microscope.

Chl fluorescence measurements

In vivo Chl *a* fluorescence parameters were determined at room temperature using either video imaging with a pulse amplitude-modulated FluorCam 700 MF device (Photon Systems Instruments, Brno, Czech Republic) or measurements with a pulse amplitude-modulated (PAM) fluorometer equipped with a PAM data aquisition system (PAM101/103 – PDA100; Heinz Walz, Effeltrich, Germany). For *Chl* video imaging wild-type plants were dark-adapted for 15 min and F_0 was measured for 3 s followed by F_m determination with a saturating light pulse of 1600 ms at 2000 µE. After a short dark-period of 60 s actinic light provided by bright-orange LED sources (λ max = 620 nm) with an intensity of 90 µE was applied for 10 min and fluorescence (F_t) was continuously recorded every 20 ms. Along of this measurement 3 saturating light pulses were given in an interval of 3 min to determine F_m' for calculation of qP and NPQ. Steady state fluorescence was taken at the end of the measurement followed by determination of F_0' in the dark for 120 s. For Chl fluorescence determination with the PAM fluorometer 10 - 15 seedlings grown on MS-medium were measured simultaneously following a protocol described earlier (Pfannschmidt et al., 2001).

The PAM system was used for determination of Chl fluorescence with PSI- or PSII-light as actinic light source. The light intensity of the PSI- and PSII-light was adjusted at 22 µE comparable to the mean light intensity in the growth chambers. PAM101 settings for the measuring light were light level 4, gain level 3 and dumping level 2. Plants were dark adapted for at least 15 minutes. After determination of $F_0'$and F_m the Chl fluorescence was measured under PSI- and PSII light sources. F_t was determined at steady state and F_0'under far red light.

The steady-state fluorescence F_s was calculated as $F_s = F_t - F_0'$. Fluorescence-quenching parameter qP (photochemical quenching) was calculated as $qP = \frac{F_m' - F_t}{F_m' - F_0'}$ (Schreiber, 1986). Fluorescence-quenching parameter NPQ (extent of non-radiative energy dissipation, relative to a dark sample) was calculated as $NPQ = \frac{F_m - F_m'}{F_m'}$ (Schreiber et al., 1997; Maxwell and Johnson, 2000). The optimal quantum yield were measured with Fluorcam 700 MF and calculated as

$\frac{F_v}{F_m} = \frac{F_m - F_0}{F_m}$ (Schreiber et al., 1997). The effective quantum yield of PSII (Φ PSII) was calculated as $\Phi PSII = \frac{F_m' - F_t}{F_m'}$ (Genty et al., 1989).

P700 absorbance measurements

16 day old *Arabdiopsis* C24 seedlings were grown in white light or adapted to PSI, PSI-II, PSII or PSII-I light as mentioned above. PSI activity was determined by measuring the light-induced *in vivo* P700 absorbance changes at 810 nm with a PAM101/PDA-100 combined with a dual wavelength emitter-detector unit (EDP700DW) (Klughammer and Schreiber, 1998). To oxidise P700 saturating far-red light of 730 nm emitted by a far-red diode (102FR) was applied for 1 min. For short-term reduction of P700 a saturating white light pulse (6000 µE) was applied for 400 ms after the first 30 s of far-red illumination and re-oxidation was followed within the next 30 s. The maximal signal difference between reduced and oxidised states of P700 (ΔA810) was taken as a measure of the photochemical capacity of P700 (Barth and Krause, 2002).

Photoinhibition experiments

Arabidopsis C24 seedlings adapted to white light, PSI- or PSII light were shifted to state I by exposition to PSI light for 30 minutes. Then the optimal quantum yield was determined using the PAM101 fluorometer (Walz, Germany). Directly after this determination plants were exposed to a strong white light (ca. 1300 µE) for 1 h inducing photoinhibition. After this plants were dark-adapted for at least 7 minutes and F_v / F_m was determined again. For the recovery process plants were placed in a continuous weak white light (60 µE) and further measurements of F_v / F_m (each after 7 minutes of dark acclimation) were performed 1 h, 3 h, 21 h and 28 h after high light application.

Plant fitness experiments

For plant fitness experiments *stn7, stn8* and *stn7/stn8* knock-out mutants (with *Arabidopsis* Col-0 as control) were grown up in the growth chambers on an earth/vermiculite mixture under alternate PSI- and PSII-light without a dark phase. In short-term experiments plants were alternately illuminated with PSI- or PSII-light every 20 minutes. For this experiment a growth cabinet was equipped with both light sources and switches between them were controlled by an electronic relais. In long-term experiments plants were shifted every 2-3 days between the light sources by exchanging the trays between the growth cabinets. When the plants started to flower each single plant was covered with an Aracon tube to facilitate seed harvesting. After

senescence and fruit ripening the seeds were collected individually for every plant and number and weight of the seeds was determined.

Acknowledgements

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Figure legends

Fig. 1: A) Plant morphology and F_s/F_m value of plants acclimated to different light quality regimes. On top phenotypic appearance of *Arabidopsis* wild-type plants acclimated to PSI, PSI-II, PSII and PSII-I light conditions shown under normal white light. Below the F_s/F_m value of the same plants as detected by parallel Chl fluorescence imaging is shown in false colours. Blue: low Fs/Fm above a threshold of 0.06; red: high F_s/F_m , upper threshold 0.17. B) Electron microscopy of chloroplasts of PSI and PSII plants. Upper micrograph: typical chloroplast of *Arabidopsis* wild type plants acclimated for 6 days to PSII light; lower micrograph: typical chloroplast of *Arabidopsis* wild type plants acclimated for 6 days to PSI light. G: grana lamellae, U: unstacked thylakoid membranes (stroma lamellae), S: starch, Pg: plastoglobuli, Sp: membrane-free stroma space. Comparable results were obtained with material harvested from PSI-II and PSII-I plants (not shown).

Fig. 2: Chl fluorescence kinetics of *Arabidopsis thaliana* WT using PSI- or PSII-light sources as actinic light. The schemes show typical Chl fluorescence traces as obtained with PSI (A) or PSII (B) plants. The light source used for illumination of plants is given in a horizontal bar on top of each trace. Upward arrow: light on; downward arrow: light off. m: measuring beam, sp: saturating white light pulse, PSI: PSI-light as actinic light source, PSII: PSII-light as actinic light source, fr: far-red diode as light source.

Fig. 3: PSI activity determined by P700 absorbance changes. Typical far-red absorption curves of P700 from differentially acclimated plants are shown in relative absorption units. ΔA_{P700} is given on the right margin of each diagram and represents the average of three absorption signals plus or minus the standard deviation. The growth light condition is shown in the upper left corner of each diagramme. fr: actinic far-red light, sp: saturating white light pulse.

Fig. 4: Photoinhibition and recovery in PSI and PSII plants. The X-axis displays the time course in logarithmic scale, the Y-axis shows F_v/F_m value as relative Chl fluorescence. Identity of the symbols is indicated in the legend. Values are the mean of three independent measurements.

Fig. 5: LTR in state transition mutants *asLhcb2* and *psaE1*
State transition mutants were grown in the standard growth programme and the LTR was determined by detecting Chl fluorescence parameters and Chl a/b ratios. The respective measured mutant is indicated on top. Values for control wild-type plants are given in the same diagramme. Description the growth light treatment is given below. Error bars indicate standard deviation. Identity of the measured parameter is given in the legend.

Fig. 6: Seed production of WT, stn7, stn8 and stn7/stn8 under various light quality conditions. Plants were grown under the different light regimes as described before, however, the plants were kept under the respective conditions until flowering and seed production. A) Seed numbers of wild-type after permanent growth in control white light, PSI-light and PSII-light. B) Response to long-term changes (shift between PSI- and PSII light every 2-3 days), C) Response to short-term changes (every 20 min). Values from mutant averages which are significantly different in comparison to Col 0 are marked by an asterisk.

Tables

	Fs/Fm		1-qP		Φ_{II}	
	PSI	**PSII**	**PSI**	**PSII**	**PSI**	**PSII**
PSI	0,014 ± 0,002	0,116 ± 0,005	0,022 ± 0,004	0,197 ± 0,014	0,742 ± 0,022	0,597 ± 0,022
PSI-II	0,01 ± 0,008	0,034* ± 0,01	0,017 ± 0,014	0,053* ± 0,015	0,731 ± 0,012	0,718* ± 0,013
PSII	0,013 ± 0,007	0,032 ± 0,009	0,025 ± 0,014	0,054 ± 0,018	0,727 ± 0,043	0,719 ± 0,043
PSII-I	0,013 ± 0,002	0,101* ± 0,008	0,021 ± 0,004	0,19* ± 0,014	0,747 ± 0,032	0,6* ± 0,037

Table 1: Photosynthetic parameters of WT under growth light sources. The respective growth light regime of the plants is given in the first column. On top the determined Chl fluorescence parameter is indicated either with PSI- or PSII-light as actinic light during the measurement. The values are means from 6 independent measurements. Standard deviation is given to the right of each value. The means were tested by a t-test for independent samples (Köhler et al., 1996). Values from plant averages which are significantly different after shifting between light sources are marked by an asterisk.

	Col 0	
	PSI	**PSII**
PSI	0,176 ± 0,04	0,262 ± 0,044
PSI-II	0,23 ± 0,06	0,183* ± 0,045
PSII	0,344 ± 0,065	0,268 ± 0,034
PSII-I	0,254* ± 0,039	0,391* ± 0,061

Table 2: NPQ values under PSI- or PSII-light in wild-type. The respective growth light regime of the plants is given in the first column. On top the light source used as actinic light is given. Values are means from 6 independent measurements. Standard deviation is given to the right of each value. The means were tested by a t-test for independent samples (Köhler et al., 1996). Values from plant averages which are significantly different after shifting between growth light sources are marked by an asterisk.

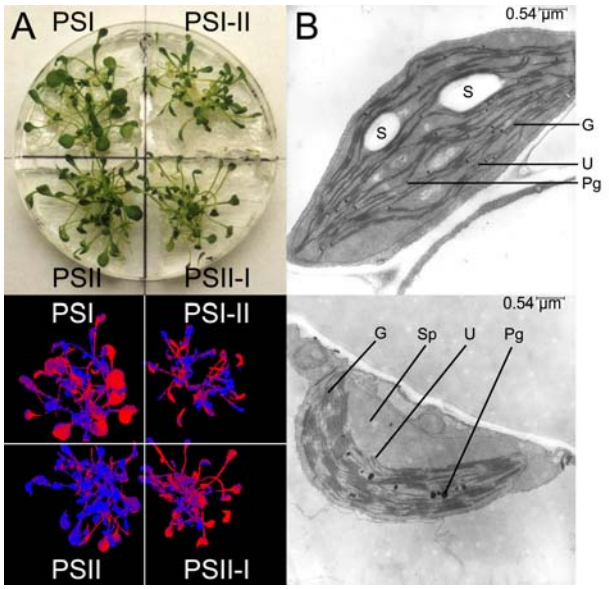
	Fs/Fm		1-qP		Φ_{II}	
	PSI	**PSII**	**PSI**	**PSII**	**PSI**	**PSII**
PSI	0.043 ± 0.012	0.217 ± 0.015	0.063 ± 0.016	0.338 ± 0.012	0.71 ± 0.003	0.493 ± 0.005
PSI-II	0.087* ± 0.005	0.239 ± 0.009	0.148* ± 0.011	0.374* ± 0.013	0.601* ± 0.016	0.452* ± 0.014
PSII	0.069 ± 0.034	0.213 ± 0.042	0.111 ± 0.052	0.325 ± 0.058	0.638 ± 0.051	0.493 ± 0.052
PSII-I	0.044 ± 0.015	0.217 ± 0.015	0.065 ± 0.022	0.339 ± 0.024	0.693 ± 0.024	0.484 ± 0.027

Table 3: Photosynthetic parameters of *stn7* under growth light sources. The respective growth light regime of the plants is given in the first column. On top the determined Chl fluorescence parameter is indicated either with PSI- or PSII-light as actinic light during the measurement. The values are means from 6 independent measurements. Standard deviation is given to the right of each value. The means were tested by a t-test for independent samples (Köhler et al., 1996). Values from plant averages which are significantly different after shifting between light sources are marked by an asterisk.

	stn7	
	PSI	**PSII**
PSI	0,125 ± 0,034	0,163 ± 0,031
PSI-II	0,201* ± 0,013	0,129 ± 0,014
PSII	0,161 ± 0,06	0,113 ± 0,046
PSII-I	0,107 ± 0,038	0,142 ± 0,026

Table 4: NPQ values under PSI- or PSII-light in the stn7 mutant. The respective growth light regime of the plants is given in the first column. On top the light source used as actinic light is given. Values are means from 6 independent measurements. Standard deviation is given to the right of each value. The means were tested by a t-test for independent samples (Köhler et al., 1996). Values from plant averages which are significantly different after shifting between growth light sources are marked by an asterisk.

Original figures



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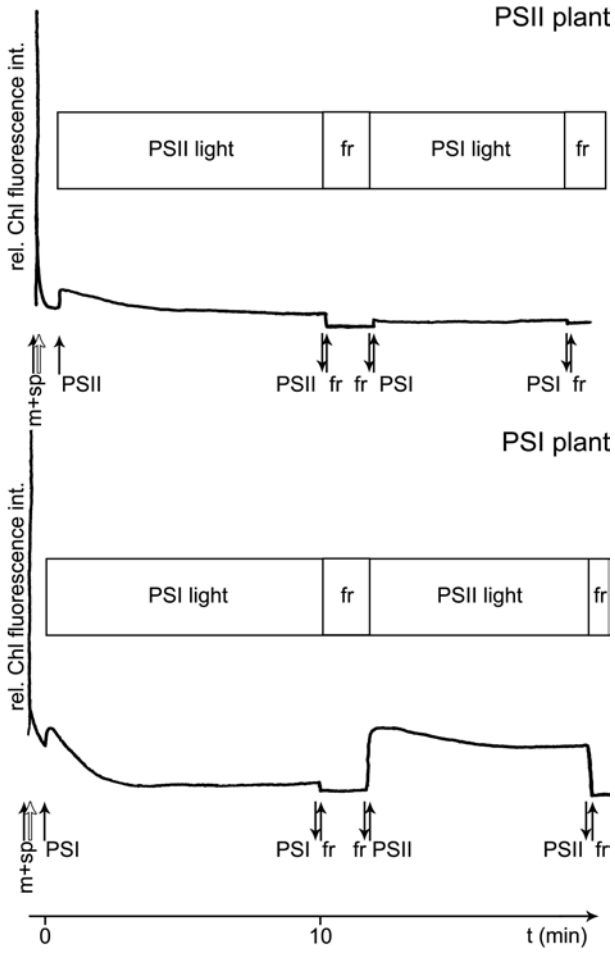

Figure 2

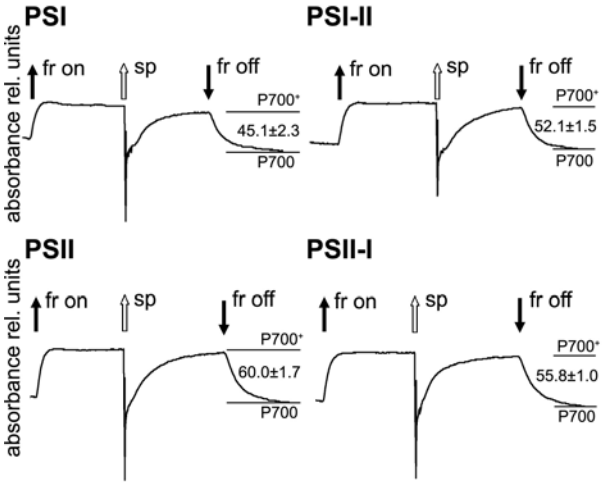

Figure 3

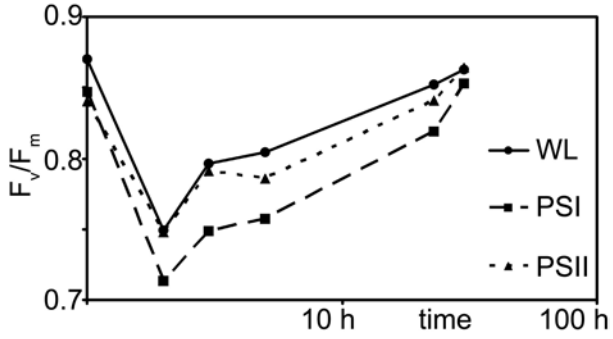

Figure 4

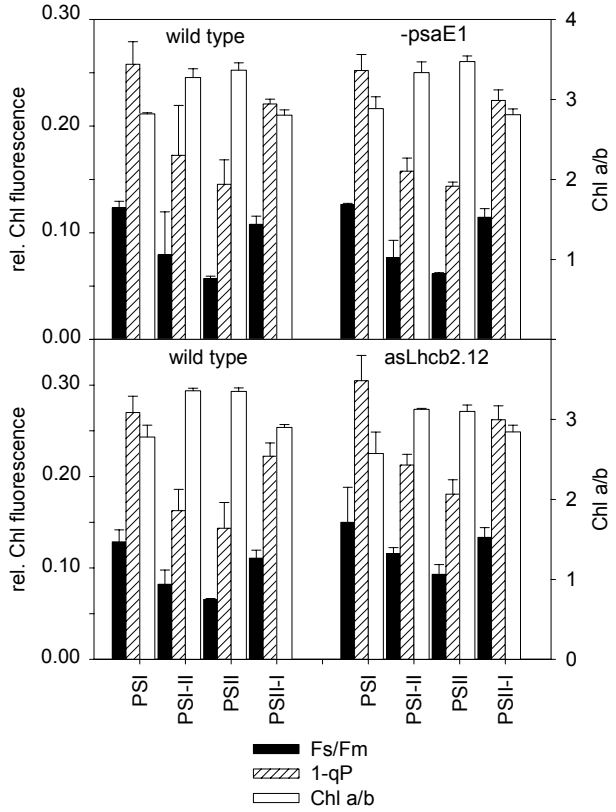
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Figure 5

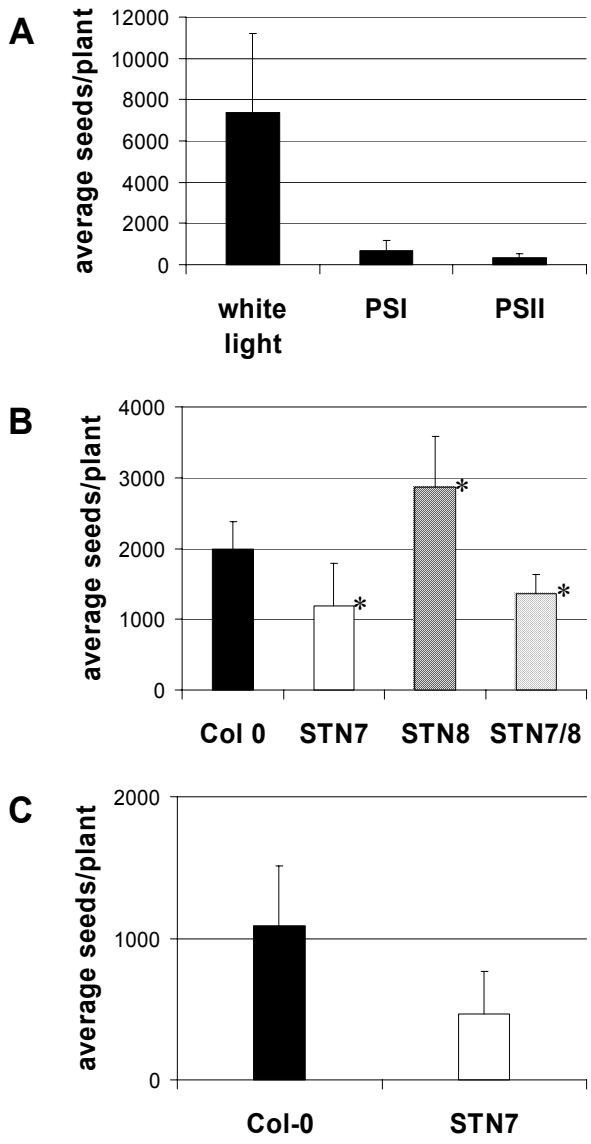
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Figure 6

5 Diskussion

5.1 PSI- und PSII-Licht – Diskussion des experimentellen Systems

PSII und PSI sind über den linearen Elektronentransport in Reihe geschaltet. Um den Elektronentransport anzutreiben, absorbieren beide Multiproteinkomplexe mittels ihrer akzessorischen Pigmente Licht. Durch den unterschiedlichen Bau der Apoproteine und der sterischen Ausrichtung der Pigmente besitzen PSII und PSI voneinander leicht verschiedene Absorptionsspektren. Diese Tatsache wird seit einigen Jahren genutzt, um die Photosysteme mit PSI- oder PSII-Licht (siehe Einleitung) unterschiedlich stark anzuregen und dadurch den Redoxzustand der Elektronentransportkette zu beeinflussen. Es zeigte sich, dass sich der photosynthetische Apparat an die jeweiligen Lichtverhältnisse anpassen kann, wobei die Art und Stärke der Anpassung von der jeweils untersuchten Spezies abhängt. Die Untersuchungen der Veränderungen auf molekularer Ebene führten zu der Entdeckung der *state transition* (siehe Einleitung). Weitere Experimente zeigten, dass zudem bei länger anhaltender Belichtung mit PSI- und PSII-Licht sich die Photosystemstöchiometrie verschob. Diese Veränderungen der Struktur des Photosyntheseapparats bezeichnet man als Langzeitantwort (LTR, aus dem Englischen: *long term response*). Bei dieser Verschiebung wird die relative Menge des jeweils lichtlimitierten Photosystems bevorzugt (Kim *et al.*, 1993; Melis, 1991). Die LTR basiert auf einer gezielten Biosynthese oder Degradation, der Photosystemuntereinheiten, die entsprechende Kontrollprozesse erfordern. Die Regulationsebene, auf die diese Prozesse abzielen, war Gegenstand langjähriger Diskussionen. Es konnte gezeigt werden, dass es Auswirkungen sowohl auf post-transkriptioneller (Deng *et al.*, 1989; Glick *et al.*, 1986) als auch auf transkriptioneller (Pfannschmidt *et al.*, 1999a; Pfannschmidt *et al.*, 1999b; Tullberg *et al.*, 2000) Ebene gibt. Die Signalwege, die diese Kontrollebenen ansteuern, sind weitgehend unbekannt. Meine Arbeit beschäftigte sich mit den molekularen Zusammenhängen in Ablauf und Kontrolle der LTR und sollte das Verständnis dieser Prozesse verbessern.

Die in unseren Experimenten verwendeten Lichtqualitäten basieren auf einer unterschiedlich starken Anregung von PSI oder PSII. Dieses Anregungsungleichgewicht konnten wir durch den Chlorophyllfluoreszenzparameter 1-qP zeigen und schlussfolgern, dass das von uns verwendete PSI-Licht die Akzeptorseite von PSII und somit PQ stärker oxidiert als PSII-Licht (Wagner *et al.*, 2006b). Dadurch ist es uns möglich, den Redoxzustand des PQ-Pools ohne Zugabe von Elektronentransport-Inhibitoren zu verändern und die dadurch ausgelöste Akklimationsreaktion zu beobachten. Auf transkriptioneller Ebene und physiologischer Ebene zeigte sich, dass ein Wechsel aus PSI- nach PSII-Licht und *vice versa* eine Verstärkung der LTR zur Folge hat. Diese Unterschiede waren größer als ein Wechsel aus Weißlicht in die entsprechende Lichtqualität (Pfannschmidt *et al.*, 1999a). Deshalb wurden die Wechselvarianten (PSI→PSII und PSII→PSI) verwendet und diese mit Pflanzen verglichen, die dauerhaft in einer Lichtqualität wuchsen. Gleichzeitig wurde dabei die

Reversibilität der beobachteten Antwort geprüft, die ein wichtiges Merkmal von Akklimationsreaktionen darstellt und gegenüber nicht revertierbaren Entwicklungsantworten abgrenzt.

Die zur Anzucht verwendeten Lichtquellen sind Schwachlicht-Lichtquellen, wodurch das Licht und die Lichtqualität die Pflanzen limitieren und damit die Akklimation und ihre Auswirkungen verstärken. Dadurch leben die Pflanzen in PSI- und PSII-Licht nahe ihres Lichtkompensationspunkts, der 1-5 $\frac{\mu mol\ Photonen}{m^2 \cdot s}$ bei Schattenpflanzen beträgt (Vogelmann, 1998).

Weiterhin zeigen die Pflanzen keine Stressreaktionen, die durch Starklicht ausgelöst werden, da der Anteil an reduziertem Glutathion nach Gabe eines Reduktions- oder Oxidationssignals sich nicht wesentlich verändert (Fey und Wagner *et al*., 2005). F_v/F_m wird häufig als Parameter verwendet, der oxidativen Stress anzeigt. Pflanzen, die an PSI- oder PSII-Licht akklimatisiert sind, zeigen keine Unterschiede zu Weißlichtpflanzen in diesem Parameter, was ein weiteres Indiz dafür ist, dass ROS als Signalüberträger bei der Lichtqualitäts-Akklimation ausgeschlossen werden können (Wagner *et al.*, 2006b).

Diurnale Effekte werden durch die Anzucht in kontinuierlicher Beleuchtung ausgeschlossen. Im Gegensatz zu vorhergehenden Arbeiten zur Lichtqualitäts-Akklimation von *Arabidopsis* (Walters und Horton, 1994) enthalten die von uns verwendeten Lichtquellen weiterhin keinerlei Blaulichtanteile. Somit werden durch Blaulicht hervorgerufene physiologische Reaktionen, wie z.B. die blaulichtgesteuerte Expression von *psaD* (Mochizuki *et al.*, 2004), vermieden.

5.2 *Arabidopsis thaliana* als Modellpflanze – ihre Vor- und Nachteile

Modellpflanzen werden ausgewählt, um spezifische Effekte oder Eigenschaften eines Teilaspektes ihrer Physiologie, ihrer Morphogenese oder ihrer Genetik mit wissenschaftlichen Methoden zu untersuchen. Dies hat den Vorteil, dass Wissenschaftler ihre Ergebnisse direkt miteinander vergleichen können, ohne dass Modellunterschiede einen Einfluss auf die experimentellen Fakten haben. Im Laufe der Geschichte der Botanik gab und gibt es eine Reihe von Modellpflanzen. So wurde *Oenothera* als Modellpflanze zur Erforschung der extranukleären biparentalen Vererbung verwendet. *Zea mays* wurde wegen ihrer Bedeutung als Kultur- und Zuchtpflanze und ihres C4-Metabolismus als Modellpflanze verwendet. Zur Erforschung des Photosyntheseapparates wurden dagegen häufig Spinat und Erbse genutzt.

Arabidopsis thaliana ist bereits seit den 40-er Jahren des 20. Jahrhunderts Objekt genetischer Studien. Bereits 1947 wurde eine Sammlung induzierter Mutanten von Reinholz (Reinholz, 1947) beschrieben. In den 70-er Jahren wurden erstmals pflanzenphysiologische genetische Experimente mit *Arabidopsis* durchgeführt und der Vorschlag unterbreitet, diese als genetischen Modellorganismus zu verwenden (Redei, 1975). Doch erst gegen Ende des Jahrhunderts wurde *Arabidopsis* zu einer nicht mehr wegzudenkenden Modellpflanze der

Grundlagenforschung in Molekularbiologie und Genetik (Meyerowitz, 2001). Der Grund hierfür sind ihre für Laborexperimente besonders geeigneten reproduktiven, molekularen und genetischen Eigenschaften.

Eine dieser Eigenschaften ist die geringe Genomgröße. So beläuft sich die Gesamtgenomgröße von *A. thaliana* auf etwa 125 MBp verteilt auf fünf Chromosomen. Weiterhin zeigt das Genom nur einen niedrigen Anteil an repetitiven Sequenzen. Dadurch war es möglich bis zum Jahr 2000 das Genom von *A. thaliana* vollständig zu sequenzieren (AGI, 2000). *A. thaliana* besitzt eine kurze Generationsdauer von nur 2 bis 3 Monaten bei einer hohen Samenproduktion pro Pflanze. Weiterhin gibt es eine Reihe von wirkungsvollen Techniken, um *Arabidopsis* zu transformieren (z.B. mittels *Agrobakterium tumefaciens* oder der *floral dip*-Technik) oder zu mutagenisieren (z.B. mit Ethylmethansulfonat (EMS)). Dies ermöglichte den Aufbau von großen Mutantenkollektionen, auf die Wissenschaftler gezielt zugreifen können. Durch leistungsstarke DNA-Sequenziermaschinen, Kartierungstechniken und digitale Speicher waren die Möglichkeiten gegeben, die erzeugten Transformanten und Mutanten zu identifizieren.

Arabidopsis zeigt jedoch auch Eigenschaften, die sich nachteilig auf biochemische Untersuchungen auswirken. So besitzt *Arabidopsis* eine geringere Biomasseproduktion im Vergleich z.B. zu *Sinapis alba* auf gleicher Fläche. Dementsprechend sind auch die Ausbeuten von Zellkompartimenten bei Zellfraktionierungen geringer. Da *A. thaliana* eine C3-Pflanze ist, kann man spezifische Fragestellungen des C4-Metabolismus nicht beantworten.

A. thaliana ist für meine Fragestellung, der Charakterisierung der LTR und LTR-Mutanten (Mutanten, die keine Langzeitantwort zeigen), somit ein idealer Organismus, da dem Phänotyp einer Mutante leichter als in anderen Organismen eine genetische Ursache zugeordnet werden kann. Dies setzt messbare phänotypische Veränderungen zur Identifikation von LTR-Mutanten voraus. Für die von uns verwendeten Lichtquellen lagen bis zum Beginn meiner Arbeit nur Daten von *Sinapis* und *Nicotiana* vor (Pfannschmidt *et al.*, 1999a; Pfannschmidt *et al.*, 1999b; Pfannschmidt *et al.*, 2001b). Deshalb führte ich Studien zur phänotypischen Charakterisierung von *A. thaliana* nach Akklimation an PSI- und PSII-Licht durch.

5.3 Einflüsse von PSI- und PSII-Licht auf *Arabidopsis thaliana*

PSI und PSII Licht haben Einfluss auf morphologischer, zellulärer und molekularer Ebene, die sich physiologisch vielfältig auswirken. Dieser Einfluß war bisher nur ungenügend charakterisiert und wurde deswegen systematisch erfasst. Ziel war die Identifizierung eines verlässlichen Parameters, über den Mutanten mit Defekten in der LTR gesucht werden können.

5.3.1 Morphologie

Pflanzen, die in PSI- oder PSII-Licht wachsen, zeigen kleinere Unterschiede in der Morphologie. Dies betrifft Blattform, Blattstiellänge und Blattquerschnitt. Gewöhnlicherweise bilden PSI-Licht akklimatisierte Pflanzen (PSI-Pflanzen) etwas kleinere abgerundete Blattflächen, längere Blattstiele und eine aufrechtere Blattstellung im Vergleich zu PSII-Licht akklimatisierten Pflanzen (PSII-Pflanzen) aus. Weiterhin besitzen PSI-Pflanzen nur eine lockere Palisadenparenchym-Zellschicht, während PSII-Pflanzen ein etwas stärker ausgeprägtes zylindrisches Palisadenparenchym zeigen (eigene unveröffentlichte mikroskopische Beobachtungen).

Diese morphologischen Effekte werden durch eine Überlagerung von verschiedenen physiologischen Reaktionen auf Lichtstärke und Lichtqualität verursacht. Beide, Lichtstärke und –qualität, werden sowohl von Photorezeptoren als auch über eine veränderte Photosyntheseeffizienz wahrgenommen und lassen sich im lebenden Organismus nur eingeschränkt voneinander trennen.

In *A. thaliana* wurde eine Vielzahl an Photorezeptoren gefunden: 5 Phytochrome (PHYA-E), 3 Cryptochrome (CRY1-3), 2 Phototropine (PHOT1 und PHOT2) und die ZTL-FKF1-LKP2 Familie (Banerjee und Batschauer, 2005). Diese nehmen u. a. Einfluss auf verschiedene Phytohormone und können so deren Effekte modulieren. Es ist bekannt, dass dadurch auch morphologische Veränderungen, wie die oben beschriebenen, hervorgerufen werden.

Die von uns beobachtbaren Akklimationsreationen sind jedoch unabhängig von den wichtigen Photorezeptoren und bekannten retrograden Signalwegen. Mutanten der Photorezeptoren PHYA, PHYB, CRY1 und CRY2 sowie Cue 1-6 und drei Gun-Mutanten zeigen Akklimation an PSI- und PSII-Licht (Fey und Wagner *et al*., 2005). Dies zeigt, dass z.B. Phytochrome keine essentielle Rolle in der LTR spielen. Andererseits wirken sich die unterschiedlichen Fernrotlicht-Anteile auf die Phytochrome und damit auf die Photomorphogenese aus, wodurch eine Überlagerung der verschiedenen Lichteffekte entsteht.

Es gibt weiterhin Indizien für Interaktionen von Signaltransduktionswegen der Photorezeptoren und für Interaktionen einzelner Photorezeptoren miteinander (Vandenbussche *et al.*, 2005). Somit geht man von einer netzwerkartigen Verknüpfung zellulärer Signalwege aus. Dass Redoxsignale einen direkten Einfluss auf die Morphologie besitzen, ist nicht wahrscheinlich, doch ist die Optimierung der Photosyntheseeffizienz das Ziel dieser morphologischen Veränderungen. Deshalb ist denkbar, dass auch die Photosynthese oder ihre Produkte Einfluss auf die Morphologie nehmen, sei dies durch Integration von Redoxsignalen in oben genanntes Netzwerk oder durch Limitierung der Produkte für den Aufbau neuer Gewebe. Da die Morphologie ein Endprodukt eines komplexen Netzwerks von physiologischen Reaktionen ist, gibt sie allerdings die primären Auswirkungen der Lichtqualitätsakklimation nur ungenügend wieder. Deshalb ist die Morphologie ungeeignet, um die Effekte der Langzeitakklimation zu untersuchen.

5.3.2 Physiologie

Morphologische Merkmale eignen sich nicht als Kriterium, um LTR-Mutanten zu identifizieren. Günstiger für eine Unterscheidung von PSII- und PSI-Pflanzen sind physiologische Merkmale, da sie die direkte molekulare Antwort auf einen Umweltreiz zeigen.

PS-Stöchiometrie

Auf Transkriptionsebene zeigte sich, dass der Transkript-Spiegel von *psaA* in PSI-Pflanzen gegenüber PSII-Pflanzen erniedrigt ist. Diese Unterschiede konnte ich auch auf der Ebene des Genprodukts von *psaA*, dem Protein PsaA, mittels einer Westernanalyse zeigen. Das Zentrumsprotein D1 des PSII hingegen zeigte nach Setzen eines Redoxsignals weder Veränderung im Apoprotein-Spiegel noch in der mRNA-Menge des zugehörigen Transkripts *psbA* (Fey und Wagner *et al*., 2005). Da D1 häufig ersetzt wird und der Reparaturzyklus mechanistisch unabhängig von der Assemblierung des PSII ist (Rokka *et al.*, 2005), prüfte ich diese Daten durch eine Western-Analyse des D2-Proteins (unveröffentlichte Daten). Dieses zweite Zentrumsprotein des PSII zeigte auch keine Veränderung im Apoprotein-Spiegel. Wir können daher schlussfolgern, dass in *A. thaliana* eine Akklimationsreaktion auf Ebene der Photosystemzentren unter unseren Lichtqualitäten stattfindet. Diese Akklimation ist jedoch einseitig, da die PS-Stöchiometrie unserer PSI- und PSII-Pflanzen durch die Menge an PSI reguliert wird. Dies bestätigt weitgehend vorhergehende Arbeiten, in denen jedoch eine antiparallele Regulation von PSI und PSII in höheren Pflanzen gezeigt worden ist (Deng *et al.*, 1989; Glick *et al.*, 1986; Pfannschmidt *et al.*, 2001b). *Arabidopsis* hat außerdem die Fähigkeit die Menge an PSII zu regulieren, wie Walters und Horton zeigen konnten. Die von ihnen gezeigte Veränderung basiert jedoch auf dem von ihnen verwendeten Lichtsystem. Dieses hatte sowohl eine höhere Lichtintensität als auch Blaulichtanteile (Walters und Horton, 1994) und spiegelt damit eher eine Lichtintensitätsantwort wider.

Veränderungen der Antennen

In den von mir durchgeführten Western-Analysen zeigte sich auch ein Einfluss auf den Proteinspiegel einzelner Untereinheiten der äußeren Antenne von PSII und PSI. Lhcb1, welches neben Lhcb2 in den LHCII-Trimeren vorkommt (Merchant und Sawaya, 2005), zeigte unter oxidierenden Bedingungen einen leicht erhöhten Spiegel. Lhca3 hingegen, welches als Homodimer mit PSI verbunden ist (Jensen *et al.*, 2003), zeigte erhöhte Mengen an Gesamtprotein unter reduzierenden Bedingungen. Diese Daten werden durch Messungen der Chl a/b-Verhältnisse gestützt, die unter reduzierenden Bedingungen ansteigen (Fey und Wagner *et al*., 2005; Pfannschmidt *et al.*, 2001b). Chlorophyll b ist, in Relation zu Chl a, häufiger in der Antenne des PS II als in der von PSI zu finden. Das Chl a/b-Verhältnis wird daher häufig als Parameter für Lichtstärke- und Lichtqualitäts-Akklimation verwendet, da es relative Antennenveränderungen und PS-Stöchiometrieveränderungen widerspiegelt. Da D1

unter den Lichtquellen sowohl im Transkript- als auch im Proteinspiegel konstant bleibt, liegt die Vermutung nahe, dass sich entweder Zusammensetzung und/oder der Antennenquerschnitt von PSII verändern. Bekräftigend für diese Vermutung ist auch die konstante Menge von D2. Die von mir gemessenen Antennen-Proteinspiegel von Lhca können durch die Mengenänderung von PSI erklärt werden. Somit zeigt sich in den von uns verwendeten Lichtqualitäten nicht nur eine Veränderung der Photosystemstöchiometrie, sondern auch eine Modifizierung der mobilen Antenne.

Ultrastruktur der Chloroplasten und der Thylakoidmembran von PSI- und PSII-Pflanzen

Das Ergebnis beider Prozesse, der LTR und der *state transition*, führt zu einer veränderten Thylakoidstruktur (Wagner *et al.*, 2006b). PSI-Pflanzen zeigen vermehrte Granastapel und weniger Stromathylakoide. *Vice versa* gilt dies für PSII-Pflanzen. Schon länger wurde vermutet, dass die Granastapel der Thylakoidmembran über nichtkovalente Wechselwirkungen der LHCII-Trimere stabil gehalten werden. Kürzlich konnte in *Arabidopsis* gezeigt werden, dass ein Fehlen von Lhcb2 keine Auswirkungen auf die Thylakoidstruktur hat (Andersson *et al.*, 2003). Die Autoren schlussfolgerten, dass Lhcb1 und Lhcb2 nicht essentiell für die Membranadhäsion sind. Sie schlossen aber nicht aus, dass eventuell andere PSII-Antennenproteine eine Rolle in der Granastapelbildung besitzen. Interessant ist hierbei die Information, dass nicht nur die LHC-Trimere von der Thylakoid-Kinase phosphoryliert werden, sondern auch Lhcb4.2 (CP29) (Tikkanen *et al.*, 2006). Man könnte spekulieren, ob dieses Protein für Wechselwirkungsprozesse in den Granastapeln mitverantwortlich ist. Man kann derzeit nicht restlos ausschließen, dass die LHCII-Trimere zumindest einen Anteil an der Membranadhäsion besitzen. So könnte die *state transition* zum Teil die Veränderungen der plastidären Ultrastruktur von PSI- und PSII-Pflanzen erklären. Da die Auswirkung auf die Thylakoidstruktur der Chloroplasten sehr stark ist, jedoch bei höheren Pflanzen maximal ein Fünftel der LHCII-Trimere an der *state transition* beteiligt ist, müssen noch andere Faktoren diese Veränderungen bestimmen. Generell stimmen die Beobachtungen mit den gängigen Modellen zur Thylakoidstruktur (Allen und Forsberg, 2001) nach wie vor überein.

Abgesehen von den Antennen spiegelt die Ultrastruktur auch die Veränderung der Photosystemstöchiometrie wider. Größere Granastapel deuten auf ein erhöhtes PSII/PSI-Verhältnis hin. Sind die Stapel kleiner und die Stromalamellen häufiger, so liegt ein verringertes PSII/PSI-Verhältnis vor (Deng *et al.*, 1989; Glick *et al.*, 1986).

In den Transmissions-Elektronen-Mikroskop-Aufnahmen von Plastiden aus PSI- und PSII-Licht ist weiterhin auffällig, dass PSII-Pflanzen normale Stärkekörner zeigen, während PSI-Pflanzen diese nicht bzw. nur deutlich eingeschränkt besitzen (Wagner *et al.*, 2006b). Dafür zeigen PSI-Pflanzen große elektronendichte Plastoglobuli.

Plastoglobuli sind elektronendichte Strukturen, die reich an Lipiden sind. Durch massenspektrometrische Analysen konnte kürzlich gezeigt werden, dass die Oberfläche der

Plastoglobuli eine Vielzahl von Proteinen enthält. Dies sind Photosystemuntereinheiten, Antennenproteine, ABC-Transporter und Enzyme verschiedener plastidärer Stoffwechselwege, z.B. der Carotenoid-Biosynthese und der Chinonsynthese. Die Zusammensetzung dieser Plastoglobuli ist variabel. Diese Daten deuten darauf hin, dass die Plastoglobuli nicht nur eine Speicherfunktion für lipophile Thylakoidbestandteile sind, sondern ebenfalls in metabolische Funktionen wie Biosynthese und der Wiederverwertung von Proteinen involviert sind (Austin *et al.*, 2006; Ytterberg *et al.*, 2006). Plastoglobuli sind mit den Thylakoidmembranen assoziiert (Austin *et al.*, 2006), wodurch es vorstellbar ist, dass die Thylakoidstrukturen aktiv durch die Plastoglobuli verändert werden.

Ebenso stellt sich die Frage, inwiefern der Stärkegehalt der Plastiden mit der Thylakoidstruktur korreliert. Plastiden aus Pflanzen mit einem Defekt in der Stärkebiosynthese, wie z.B. die adg1-Mutante (Lin *et al.*, 1988), zeigen ähnliche Thylakoidstrukturen, wie Chloroplasten aus PSI-Pflanzen (persönliche Kommunikation mit U. I. Flügge). Zusätzlich zu diesen Daten konnte beobachtet werden, dass nach der Erhöhung des CO_2-Partialdrucks die Stärkekornbildung angeregt wird (Teng *et al.*, 2006). Dabei zeigte sich ebenfalls eine Umstrukturierung der Thylakoide. Die Granastapel nahmen gegenüber normalem CO_2-Partialdruck und Stärkegehalt deutlich ab.

Interessant ist dabei der direkte Zusammenhang des Stärkemetabolismus mit dem photosynthetischen Elektronentransport. Bei Belichtung von Pflanzen kommt es durch reduziertes Thioredoxin zu einer Aktivierung der ADP-Glukose-Pyrophosphorylase (AGPase). Dieses Enzym ist ein Schlüsselenzym für den Stärkeaufbau in Chloroplasten (Geigenberger *et al.*, 2005). Zusätzlich zu dieser Redoxregulation kann es noch zur Stärkebildung bei Zugabe von Saccharose kommen. Bei Vermittlung dieses cytosolischen Signals spielt Trehalose-6-Phosphat eine entscheidende Rolle (Geigenberger *et al.*, 2005; Kolbe *et al.*, 2005; Lunn *et al.*, 2006). Es gibt Evidenzen, dass transduktionelle Redoxsignale auch einen Einfluss auf den Stärkeabbau besitzen (Sparla *et al.*, 2006).

Unsere Daten deuten stark darauf hin, dass sich PSI- und PSII-Pflanzen in unterschiedlichen metabolischen Zuständen befinden. Die beobachteten Unterschiede in der Stärkekornbildung, sowie der transkriptionellen Veränderungen der Enzyme des Metabolismus (siehe unten), führen zur Frage wie unter PSII- und PSI-Licht Stärke auf- und abgebaut wird und welche Rolle metabolische Signale bei der Umstrukturierung der Thylakoidmembran haben. Betrachtet man diese Ergebnisse in Zusammenhang mit den von uns ermittelten Kernexpressionsdaten, kann man Vermutungen über die Beziehung dieser Signalwege anstellen. Dies zu klären führt im Rahmen meiner Dissertation zu weit, zeigt jedoch auf, wie stark die einzelnen Signalwege miteinander verflochten sind.

Weiterhin stellt sich die Frage der Überlebensfähigkeit von PSI-Pflanzen. Schafft es *Arabidopsis* ohne massive Reserven an Kohlenhydraten zu wachsen und sich zu reproduzieren? Um dies zu testen, zogen wir *Arabidopsis*-Wildtyppflanzen bis zur Samenreife in PSI- und PSII-Licht an. Es zeigte sich, dass sie sowohl Wachstum zeigten, als auch Saatgut

produzierten. Unterschiede in den Saatgutzahlen waren nicht auszumachen. Daraus schlussfolgerten wir, dass den Pflanzen genügend Energie für das Wachstum zur Verfügung steht.

NPQ

PSII zeigt zusätzlich zu den veränderten Antennenprotein-Spiegeln eine weitere Anpassung auf molekularer Ebene. Diese wurde erst ersichtlich, als ich die Chlorophyllfluoreszenz von PSI- und PSII-Pflanzen unter den Anzuchtlichtquellen aufnahm (Wagner *et al.*, 2006b). Es zeigte sich, dass das nichtphotochemische *Quenching* NPQ (qE, vergleiche Einleitung) für diejenigen Pflanzen am geringsten war, die bereits zuvor an die jeweilige Lichtqualität akklimatisiert waren. Der gemessene NPQ-Parameter qE gibt den Anteil der Hitzedissipation des PSII in Relation zur Dunkelakklimation wieder. Auf molekularer Ebene wird dieser Parameter durch verschiedene PSII und thylakoidassoziierte Proteine bestimmt. Der Parameter qE ist abhängig von der Ansäuerung des Lumens. Diese Azidifizierung wird über die Protonendurchlässigkeit der ATP-Synthase reguliert. Bei höheren Lichtstärken vermindert sich diese Durchlässigkeit, wodurch der Protonengradient im Lumen ansteigt und der NPQ-Mechanismus verstärkt wird (Kanazawa und Kramer, 2002). Die Ergebnisse lassen vermuten, dass die Regulation der Durchlässigkeit nicht über eine Reduktion oder Oxidation der Thiolgruppen der γ–Untereinheit erfolgt. Diese werden vermutlich nur zur Aktivierung der ATP-Synthase bei einem Wechsel der Pflanzen aus Dunkelheit ins Licht genutzt. Der Mechanismus dieser Regulation ist derzeit noch spekulativ, wobei von einem Einfluss stromaler anorganischer Phosphate ausgegangen wird (Kanazawa und Kramer, 2002). Der Protonengradient über der Thylakoidmembran bestimmt die Aktivität von PsbS, einer Untereinheit des PSII, und von der Violaxanthin-Deepoxidase. Beide Proteine sind in den qE-Mechanismus involviert (Li *et al.*, 2004). PsbS dient der Veränderung der sterischen Anordnung von PSII-Antennenkomplexen, während die Violaxanthin-Deepoxidase der Katalyse von Violaxanthin zu Antheraxanthin dient. Die Aktivität dieser beiden Proteine führt zu einer erhöhten Energiedissipation. Für beide Proteine ist mir kein anderer kurzzeitiger Regulationsmechanismus als der Δ-pH bekannt. Ein dritter Mechanismus, der zumindest Einluss auf das qE-*Quenching* nimmt, wird für CP26 (Lhcb5) vermutet (Dall'Osto *et al.*, 2005). Letzterer ist nicht abhängig von PsbS, wurde jedoch nur in Folge von Starklichtstress gemessen. Interessant ist weiterhin, dass eine Verringerung der Lhcb2-Menge dazu führte, dass die NPQ-Kapazität kleiner wird (Andersson *et al.*, 2003), was zeigt, dass auch die Antennenkomposition einen Einfluss auf diesen Parameter nehmen kann.

Es ist deutlich, dass auch die Photochemie des PSII über längere Zeit auf das jeweilige Akklimationslicht angepasst wird. Somit optimiert die Pflanze die Quantenausbeute für die photochemische Arbeit. Dieser Optimierungsprozess scheint jedoch unabhängig von perzeptioneller Redoxkontrolle zu sein, da auch die LTR-Mutante *stn7* diese Akklimation der

PSII-Photochemie zeigt. Momentan ist noch nicht eindeutig, welcher der Mechanismen NPQ in unserem System bestimmt.

Redoxkontrolle nukleär kodierter Gene

Der Photosyntheseapparat von Algen und höheren Pflanzen ist eine Chimäre aus plastidär und nukleär kodierten Proteinen. Die Gene für die Lhc-Proteine, wie auch der Großteil peripherer Photosystemproteine, werden im Nukleus kodiert. Wir testeten den Einfluss photosynthetischer Redoxsignale auf die nukleäre Transkription durch Vergleich von PSI- und PSII-Pflanzen. Dazu modulierten wir den photosynthetischen Elektronentransport über die verschiedenen Lichtqualitäten und zusätzlich mit DCMU, einem Inhibitor von Q_B. Mittels eines *Macroarray* stellten wir fest, dass von den observierten 2661 Transkripten mit putativer plastidärer Transitsequenz (Richly *et al.*, 2003) 286 eine Regulation nach Veränderung des Redoxstatus der plastidären Elektronentransportkette zeigten (Fey und Wagner *et al*., 2005). Die signifikanten Veränderungen der Transkriptmengen der meisten Gene waren relativ gering. Die Ursache hierfür könnte im experimentellen Aufbau begründet liegen. Die RNA für die Hybridisierung wurde zwei Tage nach Setzen des Redoxsignals isoliert. In diesem Zeitraum ist die photosynthetische Akklimation weitgehend abgeschlossen (Pfannschmidt *et al.*, 2001b) und eine neue Redoxhomöostase erreicht, wodurch auch die Expressionsänderungen nicht stark ausfallen. Dennoch sind die Auswirkungen sehr komplex, wie Richly *et al.*, 2003, denen dieselben Ergebnisse vorlagen, zeigen konnten. In genannter Arbeit, die Ähnlichkeiten pflanzlicher Genexpressionsprofile von *A. thaliana* unter verschiedenen Bedingungen vergleicht, waren die Expressionsmuster der PSI- und PSII-Pflanzen verschieden vom Großteil der getesteten Bedingungen. Die größten Ähnlichkeiten fanden sich zu Experimenten unter erhöhtem Kohlendioxidgehalt (CO_2) und zu den Vergleichen von Wildtyp und den Mutanten ΔPsaN und ΔPsaO (Richly *et al.*, 2003). Interessant an diesem Ergebnis ist, dass diese beiden Untereinheiten des PSI eine Rolle beim Elektronentransport besitzen. PsaN hat eine Funktion bei der Interaktion von Plastocyanin mit PSI und PsaO ist mitverantwortlich für die Bindung von LHCII (Jensen *et al.*, 2003). Von diesen Ähnlichkeiten abgesehen zeigten sich Veränderungen der Expression für Gene, deren Produkte unterschiedlichste Funktionen übernehmen. Unter den 286 redoxregulierten Genen waren Produkte mit Funktion für den Photosyntheseapparat, für die Genexpression, für den Metabolismus, für die Signaltransduktion und anderen zu finden (Fey und Wagner *et al*., 2005). Bemerkenswert ist, dass die Gene für PSII und PSI nicht antiparallel, wie man es bei einer strikten Kontrolle erwarten würde, reguliert werden. Ebenso verändert sich die Expression nur eines Teils aller im Kern kodierten PS-Gene. Der Grund für diese Beobachtungen könnten nachgeschaltete Regulationsmechanismen auf translationeller Ebene, der Ebene des Imports und/oder der der Assemblierung sein. Der Großteil aller Lichtqualitäts-regulierter Gene zeigte eine Funktion im Metabolismus. Besonders häufig waren Enzyme des Aminosäure- und des Nukleotid-Metabolismus unter den regulierten Genen zu finden. Dies

zeigt, dass sich die photosynthetische Akklimationsantwort nicht nur auf den Aufbau des Photosyntheseapparats beschränkt. Vielmehr zeigt sie tiefe Eingriffe in den pflanzlichen Stoffwechsel.

Eine weitere bemerkenswerte Beobachtung war, dass auch Komponenten der Transkriptionsapparate von Kern (z.B. ARR9) und Mitochondrien Änderungen in ihrer Expressionsstärke zeigten. So beeinflusst die Akklimation nicht nur die plastidäre Transkription und lässt vermuten, dass die Akklimationsreaktion in einem regulatorischen Signalnetzwerk eingebunden ist. Auch neuere Daten deuten auf organisierte Interaktionen zwischen den drei genomtragenden Organellen hin (Leister, 2005; Pesaresi *et al.*, 2006).

F_s/F_m – ein physiologischer Parameter zur Charakterisierung der LTR

Die vorhergehenden Untersuchungen zeigen eindrucksvoll, wie weitreichend und komplex die Veränderungen in den Pflanzen sind. Doch ein Ausleseverfahren zur Selektion von Mutanten, die keine Lichtqualitäts-Akklimation zeigen, muss schnell und einfach zu messen sein und darf nicht in die Lebensvorgänge eingreifen. Letzter Punkt ist besonders von Bedeutung, da die Akklimation durch invasive Eingriffe gestört werden kann. Ein geeignetes Werkzeug ist die Messung der Chlorophyllfluoreszenz, da diese die PSII-Photochemie widerspiegelt ohne die Pflanze zu beschädigen. Die PSII-Photochemie lässt Rückschlüsse auf die Effizienz des Elektronentransports zwischen den beiden Photosystemen zu. Mit vergleichenden Wildtyppopulationen ist es so möglich, nichtakklimatisierte Individuen einer Mutantenpopulation zu identifizieren.

In *Arabidopsis* konnte ich vorhergehende Beobachtungen der Chlorophyllfluoreszenz von akklimatisierten *Sinapis alba* (Pfannschmidt *et al.*, 1999b) bestätigen. Diese zeigten, dass die Chlorophyllfluoreszenz nach Erreichen des Gleichgewichtzustands in PSI-Licht akklimatisiertem Senf höher ist als die von PSII-Licht akklimatisierten Pflanzen. Um relative Vergleiche anstellen zu können, entwickelten wir den Parameter F_s/F_m (vgl. Einleitung).

Bei Standardmessungen von Chlorophyllfluoreszenz-Kinetiken mit einem PAM-Fluorometer oder der Fluorcam dient eine PSII-Licht-ähnliche Strahlungsquelle als Anregungslicht. Signifikante Unterschiede zwischen PSI- und PSII-Pflanzen ergeben sich bei den Parametern F_s/F_m, 1-qP und Φ_{II}. NPQ war nicht in jedem Fall signifikant verschieden, da die Varianz dieses Parameters unter unseren Schwachlichtbedingungen zu hoch war (siehe oben).

Meine Messungen des F_s/F_m-Wertes zeigen, dass *A. thaliana* Wildtyp signifikant stärkere Chlorophyllfluoreszenz nach Akklimation an PSI-Licht aufweist (Wagner *et al.*, 2004). Zu dieser stärkeren Chlorophyllfluoreszenz kommen tendenziell erhöhte NPQ-Werte und eine Verringerung der effektiven photochemischen Quantenausbeute von PSII bei PSI-Pflanzen. Die Ursache für die erhöhten dissipativen Vorgänge von Fluoreszenz und NPQ zeigt sich am Parameter 1-qP. Der 1-qP-Parameter umschreibt den Anteil an geschlossenen

PSII-Zentren, also Reaktionskomplexen, bei denen Q_A bereits reduziert ist. PSI-Pflanzen zeigen einen doppelt so hohen 1-qP Wert wie PSII- Pflanzen unter Standardmessbedingungen. Die molekulare Ursache liegt in der oben gezeigten Verschiebung der Photosystemstöchiometrie.

Auf Basis des F_s/F_m-Parameters entwickelte ich ein Ausleseverfahren zur Identifikation von LTR-Mutanten. In einem ersten Ansatz verwendete ich ein zweistufiges Verfahren, welches ausschließlich den Parameter F_s/F_m verwendete. Eine Anwendung dieses Verfahrens führte zur Isolation mehrerer putativer LTR-Mutanten aus einer mit Neutronenbeschuss mutagenisierten Population. Die gefundenen Mutanten zeigten drei verschiedene Reaktionsverhalten in Bezug auf PSI- und PSII-Licht. Ein Typ reagierte weder auf PSI- noch auf PSII-Licht, während die beiden anderen Typen nur auf eine der beiden Lichtqualitäten mit einer Veränderung von F_s/F_m reagierten (Wagner *et al.*, 2004). Dies lässt vermuten, dass das Redoxsignal über mindestens zwei Signalwege weitergegeben wird. Leider ging der Phänotyp bei weiterer Segregation der Mutanten verloren bzw. waren die Mutanten nicht fertil. Wegen der Schwankung individueller F_s/F_m-Werte einzelner Pflanzen überlappten sich die Absolutwerte von PSI- und PSII-Pflanzen. Deshalb erweiterte ich das Selektionsverfahren um den Chl a/b-Parameter. Dies schränkte deutlich eine Selektion falschpositiver Mutanten ein.

Die erfolgreiche praktische Anwendung dieses Verfahrens konnte ich dann an der *stn7*-Mutante demonstrieren, die alle Kriterien einer LTR-Mutante aufweist (Bonardi *et al.*, 2005).

5.4 Die *stn7*-Mutante

STN7 ist ortholog zum Protein STT7 in *Chlamydomonas reinhardtii*. Diese STT7-Kinase wurde in *C. reinhardtii* als LHCII-Kinase identifiziert. Daher stammt auch die Abkürzung STT7, welche für *state transition thylakoid* steht (Fleischmann *et al.*, 1999). STN7 ist eine Herleitung aus der Abkürzung STT7. Die Aufgabe der LHCII-Kinase besteht darin, bei einer Überreduktion der Elektronentransportkette des Photosyntheseapparates die LHCII-Trimere zu phosphorylieren. Der Ursprung dieser Aktivierung ist der Redoxzustand des PQ-Pools und seines Bindungsstatus an den Cyt *b6f*-Komplex. Im Anschluss an die LHCII-Phosphorylierung findet die *state transition* statt (siehe Ultrastruktur). Durch diese Entdeckung wurde die seit dreißig Jahren gesuchte LHCII-Kinase identifiziert. Die Substratspezifität von STN7 ist derzeit Gegenstand heftiger Diskussion. Es gibt Berichte, dass sich die Funktion von STN7 und STN8, ein paraloges Protein zu STN7, ergänzen, da bei einem Ausfall der LHCII-Kinase STN7 noch immer eine gewisse Restphosphorylierung der Lhcb2 Proteine gezeigt werden konnte. Erst in der Doppelmutante *stn7/stn8* zeigte sich ein vollständiges Fehlen der Lhcb2 Phosphorylierung (Bonardi *et al.*, 2005). Entgegen diesen Beobachtungen zeigte eine andere Gruppe, dass bei Fehlen von STN7 die LHCII-Trimere vollständig unphosphoryliert vorliegen. Diese Gruppe zeigte weiterhin, dass STN7 zusätzlich

Lhcb4.2 phosphoryliert (Tikkanen *et al.*, 2006). Weitere Untersuchungen zur Substratspezifität dieser Kinasen müssen noch durchgeführt werden, um diesen Punkt zu klären.

Fest steht jedenfalls, dass bei Fehlen der STN7-Kinase in *A. thaliana* keine *state transition* mehr stattfindet. Die Pflanzen befinden sich ständig in *state 1*. Mittels des von mir entwickelten Ausleseverfahrens zeigte sich, dass die *stn7*-Mutante keine Veränderung des F_s/F_m-Wertes in PSI-, PSII- und nach Lichtqualitätswechseln zeigt. Ebenso zeigt sie keine Veränderungen im Chl a/b-Verhältnis. Aus diesen Beobachtungen wird klar, dass die *stn7*-Mutante in der Langzeitantwort defekt ist. Somit besitzt STN7 zwei Funktionen: die kurzzeitige Anpassung des Photosyntheseapparats an veränderte Lichtbedingungen und die Weitergabe dieser Information an stromale Komponenten. Diese sind noch unbekannt. STN7 ist eine Serin/Threonin-Kinase, weshalb die von ihr ausgehende Signalkette wahrscheinlich eine Phosphorylierungskaskade ist. Es ist bereits seit längerer Zeit bekannt, dass sowohl transkriptionelle als auch posttranskriptionelle Regulation in Plastiden über den Phosphorylierungsgrad einzelner Genexpressions-Komponenten erfolgen kann (Baginsky *et al.*, 1999; Liere und Link, 1997). Doch auch der stromale Redoxstatus und somit transduktionelle Redoxsignale haben einen Einfluss auf die plastidäre Genexpresseion. Eine zukünftige Identifikation weiterer Komponenten der STN7-Signaltransduktionskette wird diese komplexen Vorgänge näher beleuchten.

Da die *stn7*-Mutante defizient in der *state transition* ist, könnte dies zur Annahme führen, dass unsere Beobachtungen auf einem Ausfall der *state transition* beruhen. Um dies zu prüfen, testete ich die bekannten *state transition*-Mutanten ΔPsaE1 und asLhcb2 (Andersson *et al.*, 2003; Pesaresi *et al.*, 2002). Wir konnten beobachten, dass diese beiden Mutanten nach Akklimation an die Lichtqualitäten wildtypähnliche Veränderungen sowohl in der Chlorophyllfluoreszenz als auch bei den Chl a/b-Werte zeigten. Dies demonstriert eindeutig, dass der pure Ausfall der *state transition* nicht die LTR beeinflusst. Kurzzeitantwort und Langzeitantwort werden also über das gleiche Enzym reguliert, sind aber nicht funktionell gekoppelt. Damit ist klar, dass F_s/F_m ein exklusiver Parameter zum Anzeigen der Langzeitantwort ist und dass die *stn7*-Mutante eine LTR-Mutante ist. Daher nutzten wir die *stn7*-Mutante um die Auswirkungen der Lichtqualitätsakklimation zu testen.

5.5 Vom Nutzen der Akklimation an Lichtqualitäten

Bisherige wissenschaftliche Arbeiten zeigten, dass Pflanzen unter hohem energetischem Aufwand ihren Photosyntheseapparat verändern, um sich an die vorherrschenden Lichtbedingungen anzupassen. Der selektive Evolutionsdruck für diese Akklimation blieb jedoch lange Zeit Gegenstand der Diskussion.

In *Chlamydomonas reinhardtii* kann Starklichtstress über die *state transition* zum Teil kompensiert werden. In *Arabidopsis* ist dies nicht der Fall. Deshalb fragten wir uns, ob die LTR Vor- oder Nachteile bei einer Starklichtstress-Situation für die Pflanze bringt. Dazu

setzten wir PSI-, PSII- und Weißlichtpflanzen Starklicht aus. Vor und nach dem Starklichtstress ermittelten wir den Genty-Parameter, der ein Maß für oxidativen Stress ist. Nach dem Lichtstress wurden die Pflanzen in schwaches Weißlicht gestellt und über zwei Tage hinweg in Abständen F_v/F_m gemessen. Alle Pflanzen zeigten normale F_v/F_m-Werte vor dem Lichtstress. Starklichtstress hatte bei allen Pflanzen ein Absinken der F_v/F_m–Werte zur Folge, da D1 verstärkt durch Photooxidation beschädigt und ersetzt werden musste. PSI-Pflanzen waren auf Grund der Verschiebung der PS-Stöchiometrie, der Antennenkomplexe und dem *state 1* geringfügig stärker betroffen als PSII- und Weißlichtpflanzen. Die sich anschliessende Erholungsphase zeigte bei allen Pflanzen eine Regeneration der F_v/F_m-Werte. Diese Ergebnisse zeigen, dass der PSII-Reperaturzyklus unabhängig von der Lichtqualität voll funktionstüchtig ist und somit die LTR keine essentielle Rolle bei Stressantworten besitzt (Wagner *et al.*, 2006b).

Wir führten Chlorophyllfluoreszenz-Messungen von *Arabidopsis*-Wildtyppflanzen und *stn7*-Mutanten unter den Anzuchtlichtquellen durch. Lichtqualitäts-Akklimation sollte zu einer Optimierung der Photochemie unter dem entsprechenden Akklimationslicht führen. Für PSII-Licht war dies feststellbar, da Wildtyppflanzen, die an PSII-Licht angepasst waren, eine geringe Erhöhung des Genty-Parameters zeigten. Diese leichte Verbesserung zeigten die *stn7*-Mutanten nicht. Im Fall eines Wechsels von PSI- nach PSII-Licht verschlechterte sich sogar die Quantenausbeute des PSII. Einfluss auf die Quantenausbeute haben das NPQ (siehe oben) und die Veränderung der PS-Stöchiometrie (siehe Einleitung). Durch diese Chlorophyllfluoreszenzmessungen konnten wir jedoch keinen Vorteil von PSI-Pflanzen unter dem PSI-Licht beim Wildtyp erkennen. Die Ursache hier liegt sehr wahrscheinlich in der Messmethode begründet. Das PSI-Licht oxidiert die Elektronentransportkette sehr effektiv, hinzu kommen die schwachen Lichtintensitäten der aktinischen Lichtquellen, weshalb Unterschiede in der Variabilität der PSII-Fluoreszenz minimiert wurden. Deshalb kann man keine signifikanten Unterschiede der Parameter F_s/F_m, 1-qP und Φ_{II} ausmachen. Eine Ausnahme bildet der NPQ-Parameter, der durch Starklichtblitze bestimmt wird. Wir konnten für diesen NPQ-Parameter eine Optimierung in Bezug auf die Anzuchtslichtquelle feststellen. Interessant ist, dass die *stn7*-Mutante ebenso wie Wildtyp diese Optimierung des NPQ zeigt (Wagner *et al.*, 2006b). Diese Veränderung deutet auf eine von der LTR unabhängige Regulation dieses Parameters hin. Lichtqualitätsakklimation führt zu kleinen Unterschieden in der Energienutzung. Wir fragten uns, ob diese kleinen Unterschiede den dafür betriebenen Energieaufwand der Pflanze rechtfertigen.

Erste Messungen von *stn7*-Mutanten unter wechselnden Lichtqualitäten beschränkten sich auf Bestimmungen von Frisch- und Trockenmasse, die zeigten, dass die *stn7*-Mutante sowohl unter Stark-Schwachlichtwechseln als auch unter PSI-PSII-Lichtwechseln verringerte Mengen an Pflanzenmasse erzeugten. Die gemessenen Unterschiede verringerten sich, wenn die Bestrahlungsdauer mit der jeweiligen Lichtqualität verlängert wurde (Bellafiore *et al.*, 2005). Wir gingen einen Schritt weiter indem wir das produzierte Saatgut bestimmten. Für

eine Pflanze mit einer anuellen Lebensweise wie *Arabidopsis* besteht das evolutionäre Ziel darin, eine große Menge an fertilen Nachkommen zu erzeugen. Es konnte gezeigt werden, dass die Saatgutproduktion von *Arabidopsis* ein hervorragender Parameter zur Quantifizierung des Nutzens akklimatorischer Antworten ist (Kulheim *et al.*, 2002). Dazu zogen wir parallel Wildtypen und *stn7*-Mutanten unter kontinuierlichen oder wechselnden PSI- und PSII-Licht auf Erde an. Dadurch hatten die Pflanzen keine weitere Energiequelle als das Licht, indem sie wuchsen, zur Verfügung. Die Wechselbedingungen entsprachen 20 Minuten und zwei bis drei Tage. Im Kurzzeitwechsel vollführen die Wildtypen kontinuierlich *state transition* und im Langzeitwechsel die LTR. Die *stn7*-Mutante vollzieht weder STR noch LTR. Aus diesen Versuchen können wir eine Reihe von Schlussfolgerungen ziehen.

Wildtyppflanzen konnten bei Wachstum in kontinuierlichem PSI- und PSII-Licht fertiles Saatgut erzeugen. Die Samenmenge pro Pflanze in PSI-Licht entsprach in etwa der von PSII-Pflanzen, was für eine gleiche Energieausbeute unter beiden Lichtqualitäten spricht (siehe oben). Im Vergleich zu Weißlichtpflanzen betrug die Samenmenge pro Pflanze allerdings nur etwa 10 %. Dies demonstriert einen generellen Energiemangel unter PSI- und PSII-Licht. Vergleicht man die Entwicklung der Pflanzen unter den Wechselbedingungen, so wird deutlich, dass sich die Pflanzen in den Kurzzeitwechseln langsamer entwickelten, obwohl sie während ihrer Entwicklung die gleichen Lichtmengen erhielten wie die Pflanzen unter den Langzeitwechseln. Dies sind direkte Effekte der LTR und zeigen, dass die LTR die Pflanzen effektiver mit Energie versorgt.

Da höhere Pflanzen nur 15 % - 20 % ihrer LHCII-Trimere zur *state transition* nutzen, stellt sich die Frage der physiologischen Relevanz. Ist die *state transition* nur ein evolutionärer Überrest, da sie zum Teil den gleichen Signalweg wie die LTR nutzt? Unseren Beobachtungen nach hat die STR physiologische Relevanz, da *stn7*-Mutanten in den 20 Minuten-Wechseln weniger Saatgut bilden als Wildtyppflanzen.

5.6 Eukaryotische Transkriptionsfaktoren in Plastiden

Die LHCII-Kinase STN7 ist verantwortlich für die Langzeitakklimation des Photosyntheseapparates. In Senf konnte gezeigt werden, dass diese Langzeitakklimation eine veränderte plastidäre Transkription von PS-Genen verursacht (Pfannschmidt *et al.*, 1999a; Pfannschmidt *et al.*, 1999b). Dies legt nahe, dass auch in *Arabidopsis* die LTR über eine Modifikation der Transkription reguliert wird (Fey und Wagner *et al*., 2005). Wir vermuten, dass die LHCII-Kinase über eine Phosphorylierungskaskade Kontrolle auf transkriptioneller Ebene ausübt (Fey *et al.*, 2005). Es ist aus *in vitro*-Experimenten bekannt, dass sowohl die plastidäre Transkription als auch Translation über Phosphorylierung und transduktionelle Redoxsignale reguliert werden können (Link, 2001). Da STN7 eine Serin/Threonin-Kinase ist, liegt nahe, dass das Signal durch Phosphorylierung weitergegeben wird. Doch wohin geht dieses Signal? Ein aktuelles Modell sieht vor, dass in Analogie zu bakteriellen Systemen das Redoxsignal über eine Phosphorylierungskaskade auf Transkriptionsfaktoren übertragen wird

(Allen, J. F., 1993; Allen, John F., 1993). Was ist die Identität solcher Transkriptionsregulatoren? Der plastidäre Transkriptionsapparat besteht soweit bekannt aus zwei verschiedenen Enzymen. Neben der bakterienähnlichen plastidenkodierten RNA-Polymerase (PEP) kennen wir noch die phagenähnliche kernkodierte RNA-Polymerase (NEP).

Die PEP ähnelt sehr stark Polymerasen, die wir aus Bakterien kennen. Differentielle Genexpression in Bakterien wird durch zusätzliche Faktoren, die Sigma-Faktoren, vermittelt. Diese sorgen für die promotorspezifische Bindung der bakteriellen RNA Polymerase. In *A. thaliana* wurden sechs Sigmafaktoren gefunden. Diese sind nukleär kodiert und zeigen eine Entwicklungs- oder Gewebs-spezifische Aktivität (Kanamaru und Tanaka, 2004). Untersuchungen einzelnder *knock-out*-Linien zeigen jedoch keinen oder nur einen schwachen Phänotyp bei Kotyledonen, während adulte Pflanzen normale Phänotypen zeigen (Ishizaki *et al.*, 2005). Entweder durch andere Sigma-Faktoren oder durch noch nicht identifizierte Transkriptionsfaktoren scheinen die *knock-out*-Mutanten die fehlenden Sigma-Faktoren zu kompensieren. Es gibt zwei Ausnahmen: Sigmafaktor 2 (Sig2) und Sigmafaktor 5 (Sig5). Sig2-*knock-out*-Linien zeigen einen starken Phänotyp und Störungen in der Plastidenentwicklung. Für Sig5 wurde eine spezifische Kontrolle in der Vermittlung von Blaulichtsignalen gezeigt (Mochizuki *et al.*, 2004). Dennoch erklären diese wenigen Faktoren nicht die Vielfalt der Promotornutzung, die in der plastidären Transkription gezeigt wurde (Meng *et al.*, 1991; Pfannschmidt und Link, 1997).

Die plastidären Polymerasen assemblieren mit der plastidären DNA und vielen anderen kernkodierten Proteinen zu einem Superkomplex (TAC) (Pfalz *et al.*, 2006). Da diese einzelnen an der Transkription beteiligten Faktoren in kleinsten Mengen in den Plastiden vorkommen, konnten bisher nur sehr wenige in den Plastiden nachgewiesen werden (Wagner und Pfannschmidt, 2006). Aus diesem Grund wählten wir einen bioinformatischen Ansatz, um putative plastidäre Transkriptionsfaktoren zu finden, die in die redoxregulierte plastidäre Signaltransduktion involviert sein können. Nukleär kodierte Proteine werden mittels einer N-terminalen Signalsequenz an ihren Bestimmungsort transportiert. Solche Transitpeptide besitzen eine oder mehrere Grundstrukturen. Mit Kenntnis dieser kann man Vorhersagen über den Bestimmungsort eines Proteins über spezielle Algorithmen erstellen. Mittels verschiedener Vorhersageprogramme durchsuchte ich zwei Transkriptionsfaktoren-Datenbanken nach Proteinen, die in Plastiden importiert werden können. Nach restriktiver Analyse der Daten der Vorhersageprogramme ergab sich eine Anzahl von mindestens 48 putativen eukaryotischen Transkriptionsfaktoren. Solch eine hohe Anzahl könnte die flexiblen plastidären Transkriptionsvorgänge erklären, besonders da eukaryotische Transkriptionsfaktoren als Heterodimere arbeiten können. Weiterhin zeigte sich, dass eine Vielzahl dieser Faktoren eukaryotischen Ursprungs sind und dass es auf Ebene der plastidären DNA *cis*-Elemente gibt, an die diese Transkriptionsfaktoren binden könnten. Zum Nachweis

einer Interaktion der postulierten redoxkontrollierten Phosphorylierungskaskade mit den Transkriptionsfaktoren bedarf es weiterer Arbeiten, die bereits im Gange sind.

5.7 Aktuelles Modell der Akklimationsreaktion von *A. thaliana* an PSI- und PSII-Licht

Die von mir gewonnenen Daten ermöglichen es, ein deutlich genaueres Bild über Hergang und Folgen der Akklimation von *A. thaliana* an PSI- und PSII-Licht zu erstellen. Die Akklimation ist dabei von der Art des Redoxsignals abhängig und führt zu komplexen Veränderungen der Struktur und Funktionalität des Photosyntheseapparates, der Thylakoidmembran und des Metabolismus. Ein Modell der molekularen Regulation könnte wie folgt aussehen (siehe auch Abb. 4).

Ein Reduktionssignal wird durch den Wechsel aus PSI- nach PSII-Licht erzeugt (PSI-Licht→PSII-Licht). Durch diesen Wechsel wird PSII bevorzugt angeregt und die Elektronentransportkette durchreduziert. Dieser Effekt wird durch die vorhergehende Akklimation an PSI-Licht noch verstärkt. Die Überreduktion führt zur Aktivierung der thylakoidassoziierten Kinase STN7. Die aktive STN7-Kinase ist eine Serin/Threonin-Kinase, die direkt oder indirekt Lhcb1, Lhcb2 und Lhcb4.2 phosphoryliert (Depège *et al.*, 2003; Tikkanen *et al.*, 2006). Diese Phosphorylierung löst die *state transition* aus und etwa ein Fünftel der LHCII-Trimere wandert zu PSI, wodurch die Pflanze in *state 2* übergeht. Die LTR ist unabhängig von der *state transition* (Wagner *et al.*, 2006b). Die aktive LHCII-Kinase STN7 gibt zu stromaler Seite das Signal für die LTR an ein noch unbekanntes Substrat weiter. Es ist ein Protein TSP9 (*thylakoid soluble phosphoprotein of* 9 kDa) bekannt, welches thylakoidassoziiert ist und bei Beleuchtung der Pflanzen phosphoryliert wird. Nach Phosphorylierung löst sich ein Teil dieses TSP9 (etwa 15 %) von der Membran und diffundiert in das Stroma der Chloroplasten (Carlberg *et al.*, 2003). TSP9 enthält im C-Terminus eine basische Domäne, die eventuell als DNA-Bindedomäne fungieren und damit das Signal auf Transkriptionsebene weiterleiten könnte. Vorhergehende Arbeiten zeigen, dass Komponenten des Transkriptionsapparates, insbesondere die Sigma-Faktoren, durch Phosphorylierung reguliert werden (Link, 2001, 2003). Es ist damit sehr wahrscheinlich, dass eine Phosphorylierungskaskade den plastidären Transkriptionsapparat steuert. Für eine genspezifische Steuerung reichen die bekannten sechs Sigma-Faktoren allerdings nicht aus. Biochemische Daten (Sato *et al.*, 1993) sowie meine eigenen bioinformatischen Ergebnisse legen nahe, dass Plastiden eine Vielzahl noch unbekannter eukaryotischer Transkriptionsfaktoren enthalten, die genau dies vermitteln könnten. Wir vermuten, dass nukleär kodierte TF die Transkription von *psaA* spezifisch verstärken, was höhere *psaA*-Transkriptmengen und einen erhöhten Genproduktspiegel zur Folge hat (Fey und Wagner *et al*., 2005). Die gesteigerte Biosynthese von PSI-Apoprotein dient als Schrittmacher für eine vermehrte PSI-Assemblierung und könnte somit Ursache für die Vermehrung der Stromathylakoide unter PSII-Licht sein. Durch die Neusynthese von PSI wird die PS-

Stöchiometrie zugunsten von PSI verschoben und dadurch die Überreduktion der Elektronentransportkette vermindert.

Beim Setzen eines Oxidationssignals (PSII-Licht→PSI-Licht) wird die Elektronentransportkette oxidiert (Wagner *et al.*, 2006b). Dadurch wird die Aktivität der STN7-Kinase stark vermindert. Durch konstitutiv arbeitende LHCII-Phosphatasen sinkt der Phosphorylierungsgrad der LHCII. Dadurch werden diese wieder zu PSII hin verschoben und die Pflanzen gehen in *state 1* zurück. Durch die verminderte STN7-Aktivität wird aber auch die Phosphorylierungskaskade, die die psaA Transkription fördert, abgeschaltet. Dadurch sinken die *psaA*-Transkriptmengen ab, wodurch sich die Rate der PsaA Neusyntheseder vermindert. Als Folge verändert sich die PS-Stöchiometrie zum Vorteil von PSII. Interessanterweise ist das *psaA*-Transkript eines der plastidären Transkripte mit kurzer Lebensdauer (Rapp *et al.*, 1992), weswegen gerade hier eine transkriptionelle Steuerung Sinn macht.

Dieses Modell funktioniert nur, wenn wir davon ausgehen, dass es zusätzlich zur redoxregulierten Transkription plastidärer Gene ein Plastidensignal gibt. Dieses muss retrograde Kontrolle auf nukleäre PS-Gene ausüben, da deren Genprodukte für eine PSI-Assemblierung benötigt werden. Tatsächlich lassen unsere Daten solch eine retrograde Regulation erkennen. Wir wissen zwar noch nicht, wie dieses Signal weitergeleitet wird, doch mit Hilfe der von mir identifizierten LTR-Mutante ist es zukünftig möglich dieser Fragestellung gezielt nachzugehen. Unser Modell eignet sich damit ideal, um Lichtqualitätsakklimationen höherer Pflanzen zu untersuchen und ihre molekularen Ursachen zu identifizieren.

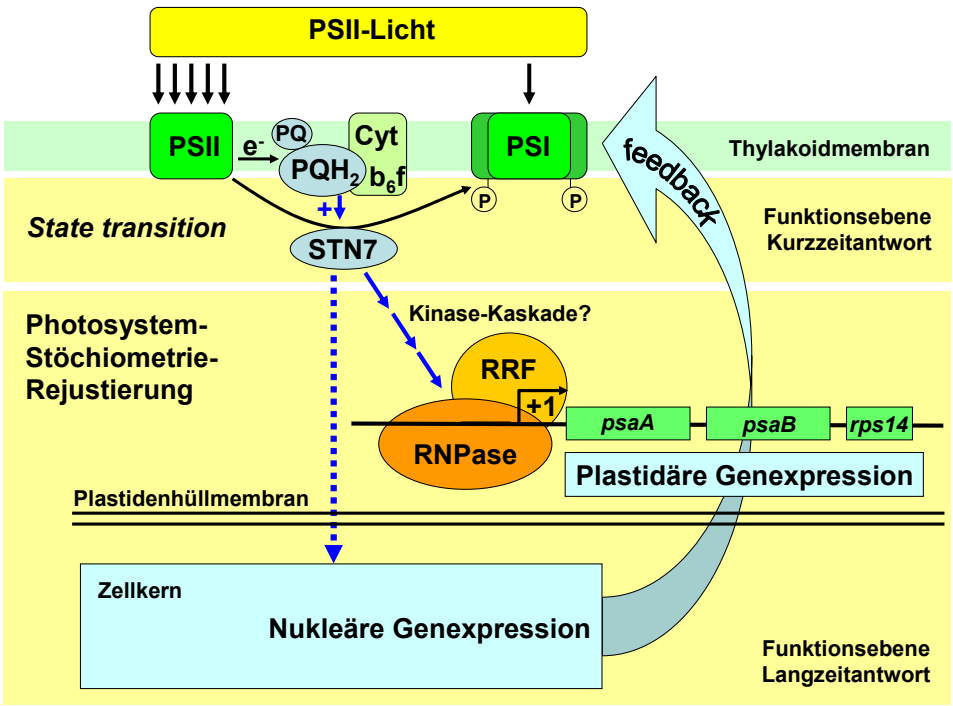

Abb. 4: Modell der redoxregulierten Lichtqualitäts-Akklimation. Entnommen aus Wagner *et al.*, 2006a. Abkürzungen: RRF – *redox responsive factor*, RNPase – RNA-Polymerase.

6 Zusammenfassung

Höhere Pflanzen sind sessile Organismen, daher sind sie den jeweiligen Umweltfaktoren ihres Habitats ausgesetzt. Zu solchen Faktoren gehören Wasserverfügbarkeit, Kohlendioxidkonzentration, Nährelementverfügbarkeit und Licht. Das Licht kann in Quantität und Qualität je nach Habitat verschieden sein. Vergleicht man das Lichtspektrum einer offenen Wiese und das eines Waldes, so zeigt sich neben der verringerten Lichtintensität im Wald auch eine Veränderung der Zusammensetzung des Spektrums (Wagner *et al.*, 2006a). Pflanzen reagieren auf solche Unterschiede vor allem durch Akklimationsreaktionen auf physiologischer und molekularer Ebene. Die Bedeutung der Lichtqualitäts-Akklimationsreaktion zeigten Fertilitätsversuche mit Wildtyppflanzen und *stn7*-Mutanten. Pflanzen, die sich akklimatisieren, erzeugen mehr Nachkommen und haben somit einen evolutionären Vorteil, da sie flexibler in verschiedenen Habitaten auf abiotische und biotische Faktoren reagieren können (Wagner *et al.*, 2006b). Diese Akklimationsreaktionen führen z.B. zu einem Umbau des photosynthetischen Apparates, um das Lichtspektrum optimal auszunutzen. Der Rezeptor für die Erkennung von spektralen Veränderungen ist, neben den Photorezeptoren, der Photosyntheseapparat selbst. Dieser erzeugt ein Redoxsignal, dass ihn in Form eines *feed-back* Mechanismus reguliert. Wie dieses vom photosynthetischen Apparat erzeugten Redoxsignal weitergeleitet wird, ist weitestgehend unbekannt.

Um diese Signalweitergabe zu erforschen, verwendete ich verschiedene Lichtqualitäten, die solche photosynthetischen Redoxsignale erzeugen, indem sie eines der beiden Photosysteme der Thylakoidmembran bevorzugt anregen (Wagner *et al.*, 2004). Mit diesem experimentellen System untersuchte ich die Reaktionen der Modellpflanze *Arabidopsis thaliana* und fand Anpassungsreaktionen auf molekularer, physiologischer und zellulärer Ebene (Fey und Wagner *et al*., 2005; Wagner *et al.*, 2006b). Verschiedene daraus resultierende physiologische Parameter wurden getestet und aus diesen ein Selektionsverfahren entwickelt, mit welchem man Mutanten mit einer Defizienz in der Akklimation an Lichtqualitäten identifizieren kann. Dieses Verfahren konnte ich erfolgreich anwenden und zeigen, dass eine Mutante, der das STN7-Protein fehlt, alle Parameter einer Akklimations-Defizienz erfüllt (Bonardi *et al.*, 2005). Da die STN7-Kinase eine zweite Funktion, die sogenannte *state transition*, besitzt, testete ich andere Mutanten, die einen Defekt in dieser biochemischen Funktion aufweisen. Es zeigte sich, dass diese *state transition*-Mutanten nicht in der Langzeitakklimation betroffen waren (Wagner *et al.*, 2006b). Da diese Mutanten in Komponenten betroffen sind, die direkt in die Umlagerung der Lichtsammelkomplexe involviert sind, kann man davon ausgehen, dass der Signalweg für die Langzeitakklimation direkt von der STN7-Kinase oder eines der direkt folgenden Enzyme ausgeht. Damit gelang es mir erstmals zu zeigen, dass die biochemischen Modifikationen eng mit dem längerfristigen Umbau des photosynthetischen Apparates verbunden sind. Die Identifikation der STN7-Kinase macht wahrscheinlich, dass das Rodoxsignal über eine Phosphorylierungskaskade weitergegeben wird. Unsere Daten zeigen, dass die *psaA*-Transkriptmenge durch den Redoxzustand des Plastochinonpools betroffen ist. In Verbindung mit früheren biochemischen Daten ist es höchst wahrscheinlich, dass die postulierte Phosphorylierungskaskade Komponenten des Transkriptionsapparates reguliert und diese, durch die STN7-Kinase aktiviert, vermehrt *psaA* transkribieren (Fey *et al.*, 2005). Weiterhin

konnte ich zeigen, dass es möglicherweise mehr eukaryotische Transkriptionsfaktoren in Plastiden gibt, als bisher vermutet wurde. Diese könnten die Effekte vielfältiger Promotornutzung und differentieller Genexpression erklären, die in Plastiden gefunden wurden (Wagner und Pfannschmidt, 2006). Damit stellen sie mögliche regulatorische Komponenten der Phosphorylierungskaskade dar.

Ich konnte zeigen, dass auch die Apoproteinspiegel von PsaA mit dem Anstieg des *psaA*-Transkripts korrelieren (Fey und Wagner *et al*., 2005). Diese Erhöhung des PSI-Zentrumsproteins dient als Schrittmacher für eine verstärkte Assemblierung dieses Photosynthesekomplexes. Dies macht die Existenz eines retrograden Plastidensignals sehr wahrscheinlich, da PSI-Untereinheiten im Nukleus kodiert sind und diese zur Assemblierung von PSI benötigt werden. Wir konnten zeigen, dass ein photosynthetisches Redoxsignal Einfluss auf die Expression nukleär lokalisierter Photosynthesegene zeigt (Fey und Wagner *et al*., 2005). Damit ist unser System ideal zur weiteren Identifikation und Charakterisierung von Komponenten der perzeptionellen redoxkontrollierten Signaltransduktion. Ein Verstehen um deren Natur und Interaktionen dieser Komponenten ermöglicht es zukünftig die regulatorischen Vorgänge der Plastiden besser zu verstehen und die evolutionären Ursprünge dieser Regulationswege zu klären.

Synopsis

Higher plants are sessile organisms. Therefore they are exposed to their habitat and have to deal with fluctuations of environmental factors. The main factors are the availability of water, CO_2, nutrients and light. Light can change in intensity and quality in one habitat and between different habitats. Often both, light quantity and quality are changed. This could be seen by comparing the light spectra of an open meadow and in the shadow of a forest (Wagner *et al.*, 2006a)*. Plants acclimate to such differences at the molecular and at the physiological level. The impact of acclimation responses is shown by my fertility experiments. In these the plants lack of an acclimation response (especially in the* stn7 *mutant) represents a disadvantage in fitness parameters. So acclimation to different light qualities is an evolutionary advantage because plants are more flexible to changing limits of biotic or abiotic factors like light and can colonize a wider range of habitats* (Wagner *et al.*, 2006b)*. During light quality acclimation plants change their photosynthetic apparatus to optimize the usage of the incident light quality. Beside photoreceptors, it has been observed that the photosynthetic apparatus itself is a light quality and -quantity receptor. It was shown that photosynthesis regulates itself by a feed-back mechanism. The components and the mechanisms of this light quality signaling pathway are largely unknown.*

To investigate this topic I used different light quality sources which produce photosynthetic redox signals by using the different excitation preferences of the two photosystems (Wagner *et al.*, 2004)*. The system is a useful tool to analyse the acclimation responses of* Arabidopsis thaliana*. The plant exhibited changes at molecular, cellular and physiological level* (Fey und Wagner *et al*., 2005; Wagner *et al.*, 2006b)*. Parameters which characterize differences in acclimation were used to develop a screening procedure for mutants which have deficiencies in their response to different light qualities. Successfully I practiced this procedure to identify a mutant which exhibits no response in all tested parameters for light quality acclimation. The mutant is deficient in the STN7 protein* (Bonardi

et al., 2005). *This is a thylakoid associated kinase and functions in state transitions as a LHCII kinase. Additional I tested other mutants which show no state transition at all. These could perform long term light quality acclimation* (Wagner *et al.*, 2006b). *These latter mutants are knock-out lines of components which are directly involved to the re-arrangement of the light harvesting complexes. Therefore the redox signaling pathway is branching at the point of STN7 or at an enzyme acting after STN7 and before the phosphorylation of LHCII trimers. These data indicate the close relationship of short-term and long-term response. Because STN7 is a kinase it is very likely that the redox signal is transduced* via *a phosphorylation cascade. Our data reveal the impact of photosynthetic redox control on* psaA *transcript level. Former data indicate a regulation by phosphorylation at the transcriptional level of* psaA (Fey *et al.*, 2005). *It is likely that the origin of this phosphorylation is the STN7 kinase. In addition to these data I could find evidences for a much higher number of eukaryotic transcription factors in plastids assumed before. These could explain the differences in promoter usage and different gene expression in plastids* (Wagner und Pfannschmidt, 2006). *Additional these factors could be novel regulation targets for redox controlled signaling pathways.*

The apoprotein level of PsaA is changed in the same manner like the psaA *transcript* (Fey und Wagner *et al*., 2005) *and could work as a pace maker of PSI assembly. Additional components are required for this formation. Many of them are encoded in the nucleus. This requires the existence of a redox controlled retrograde pathway. We could show that perceptional photosynthetic redox signals have an impact to nuclear transcription of photosynthetic genes* (Fey und Wagner *et al*., 2005).

These data and the identification of the stn7 *mutant shows that our model system is a useful tool to investigate single components of the perceptional redox controlled signaling pathways. The knowledge of nature and interaction of these components is essential for the understanding of regulatory pathways and their evolutionary origin.*

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8 Eigenständigkeitserklärung

Ehrenwörtliche Erklärung zur Anfertigung der Dissertation

Entsprechend der Promotionsordnung der Biologisch-Pharmazeutischen Fakultät der Friedrich-Schiller-Universität Jena erkläre ich hiermit, dass ich die vorliegende Arbeit selbständig und nur unter Verwendung der angegebenen Hilfsmittel und Literatur angefertigt habe.

Personen, die bei der Auswahl und Auswertung des Materials und der Erstellung der Manuskripte behilflich waren sind am Beginn eines jeden Manuskripts angegeben.

Die Hilfe eines Promotionsberaters wurde nicht in Anspruch genommen.

Die vorgelegte Arbeit wurde bislang weder an der FSU-Jena, noch an einer anderen Hochschule als Dissertation eingereicht.

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Raik Wagner
Jena, 06. Dezember 2006

9 Lebenslauf und wissenschaftlicher Werdegang

Schule

29.06. 1976	Geburt in Marienberg (Erzgeb.)
01.09. 1983 – 31.08. 1991	Polytechnische Oberschule „Johann Wolfgang von Goethe“ in Olbernhau
01.09. 1991 – 31.08. 1992	Erweiterte Oberschule in Olbernhau
01.09. 1992 – 17.06. 1995	Städtisches Gymnasium Olbernhau
1993-1995	Belegung der Leistungskurse Biologie und Mathematik am Städtischen Gymnasium Olbernhau
17.06. 1995	Erlangung der Hochschulreife (Abitur)

Wehrdienst

01.07. 1995 - 30.04. 1996	Grundwehrdienst in Hemau

Wissenschaftlicher Werdegang und Liste wissenschaftlicher Vorträge

01.10. 1996 – 30.09. 1998	Grundstudium im Studiengang Biologie (Diplom) an der Biologisch-Pharmazeutischen Fakultät der „Friedrich-Schiller-Universität“ in Jena
ab 01.10. 1998	Hauptstudium im Studiengang Biologie (Diplom) an der FSU-Jena
22.08. 2002	Abschluss der Diplomprüfung
22.08. 2002 - 27.08. 2002	Besuch der Botanikertagung in Freiburg i. Br. Vortragstitel: *A new screen for Arabidopsis mutants with defects in redox signalling pathways*
27.08. 2002	Verleihung des Titels „Diplombiologe“
30.09. 2002	Exmatrikulation als ordentlicher Studierender
01.10. 2002	Immatrikulation als Promotionsstudent an der FSU Jena
01.10. 2002 – 30.09. 2003	Landesgraduiertenstipendiat
01.10. 2003 –	Wissenschaftlicher Mitarbeiter in der Arbeitsgruppe von PD Dr. Thomas Pfannschmidt
26.07. 2004	Vortrag im Bot. Institut in Münster im Rahmen einer Kooperation Vortragstitel: Einfluss der Lichtqualität auf Proteinkomplexe der Thylakoidmembran bei Senf und Tabak
05.09. 2004 – 10.09. 2004	Besuch der Botanikertagung in Braunschweig Vortragstitel: *Light quality effect on structure and function of the photosynthetic apparatus of Arabidopsis thaliana*
08.10. 2004	Vortrag auf der „Photosynthese Nord-West“ Tagung in Münster Vortragstitel: *Photosynthetic acclimation to different light qualities in Arabidopsis thaliana*

22.10. 2004 - 24.10. 2004	Gemeinsame Organisation und Durchführung eines Doktorandentreffens der Forschergruppe FOR 387 mit Katharina Bräutigam
23.10. 2004	Vortrag auf dem Doktorandentreffen der FOR 387 in Jena Vortragstitel: *Double-screen for mutants which lack a response to different light qualities in Arabidopsis thaliana*
19.01. 2006	Vortrag auf dem „Middle East Plant Molecular Biology Meeting“ in Dresden Vortragstitel: *The thylakoid associated kinase* STN7 *regulates long-term light quality acclimation in Arabidopsis thaliana*
09.03. 2006	Vortrag auf dem Doktorandentreffen in Dabringhausen Vortragstitel: Einfluss der STN7-Kinase auf die Stöchiometrie der Photosysteme in *Arabidopsis thaliana*

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